

Improving the larvae acceptance rate and morphometric characteristics of queen *Apis cerana javana* by grafting larvae at different ages

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Abstract. Ustadi, Ikhsan N, Junus M, Suyadi. 2021. Improving the larvae acceptance rate and morphometric characteristics of queen *Apis cerana javana* by grafting larvae at different ages. *Biodiversitas* 23: 166-170. This study was aimed to evaluate the effect of different ages of grafted larvae on the acceptance rate and morphometric characteristics of queen *Apis cerana javana* Fabr. The total of 72 larvae were 1, 2, and 3 days old and was reared in 7 colonies with 3 treatments (larvae age) and 6 replications. The morphometrics studied consisted of body length, emergence weight, head length, head width, thorax length, thorax width, fore wing length, fore wing width, abdomen length, and abdomen width. The results showed that the different ages of grafted larvae had a highly significant on body length, emergence weight ($P < 0.01$), significant on abdomen length ($P < 0.05$), but not on head length, head width, thorax length, thorax width, fore wing length, fore wing width, abdomen width, acceptance rate. However, the higher acceptance rate was 73.33% to be found in 1 day old of larvae than in 2 and 3 days old. The age of grafted larvae of 1 day old has a higher body length, emergence weight, and abdomen length ($P < 0.05$) than in 2 and 3 days old of larvae. Thus, it can be concluded that the age of grafted larvae of 1 day old was the best age to queen rearing of *Apis cerana javana*.

Keywords: Grafted age, larvae, morphometrics, queen rearing

INTRODUCTION

Honeybee of *Apis cerana* Fabricius (1973) was included in the group Indo-Malayan *cerana*, which area started from southern Thailand, through Malaysia, and Indonesia. Three subclusters from Indo-Malayan group: (a) Palawan (Philippines) and Borneo bees; (b) Malay Peninsula, Sumatera, and some Sulawesi bees; and (c) Indonesia including Java, Bali, Papua, some Sulawesi, and Sumatera bees (Radloff et al. 2011). In Indonesia, the *Apis cerana* is mostly using a traditional hive to be keeping them, however in recent have been using a box hive. In addition, some problems of beekeeping of *Apis cerana javana* in Indonesia (Islands of Java, Bali, Nusa Penida, and Sumbawa) consists of the queen bee rearing is minimum, bees absconding, bee space understanding, not standardized of a beehive, the honey moisture content is high which was impacted on the fermentation process (Schouten et al. 2019).

A recent study has been reported that the good performance of the honeybee colonies was determined by the queen bee quality, especially the productivity to produce eggs (Szawarski et al. 2019). In addition, it was also affected by the genetic, defensiveness, tolerance or resistance from parasite and disease, colonies growth rate, and the feed availability for honeybees. However, when honeybees keeping, usually there is a queen bee that is less of productivity which is characterized by less eggs production, laying irregular eggs in the nest, such as in the one nest contains two or more eggs, the less of pheromone

production (Koeniger et al. 2011). The queen less of productivity must be replaced with higher productivity to increase the bee colonies' productivity in the apiary. In addition, several physical parameters which were supported the productivity of queen bees, such as emergence weight, thorax (length and width), head, and abdomen length (Okuyan and Akyol 2018). Furthermore, the productivity of queen bees was also limited by age, where their age ranged from 2 to 4 years. The nest that contains queen bee larvae usually contains royal jelly much more than for workers and drones larvae, which was impacted on the faster growth and bigger body size (De Souza et al. 2018). Pollen is essential for sexual reproduction (Nuriyah et al. 2021).

A recent study showed that the queen bee could be made from 3 days old of larvae using a grafting technique (Okuyan and Akyol 2018), but their quality was decreased when the larvae age was increased (Kuntadi 2013) and it is related to time for larvae stage completed. Furthermore, the old of larvae require a shorter time in larval stages and they consume a small amount of royal jelly (Kuntadi 2013). Kuntadi (2013) was reported that the queen bee of *Apis cerana*, which was produced by 1 day old larvae using a grafting technique, had a heavier body weight and egg production was higher than 2 days old larvae. Furthermore, it was reported that queen bees of *Apis mellifera meda* were reared from 1-day old larvae has a bodyweight heavier than 2 days old larvae and the lightest in 3 days old larvae (Mahbobi et al. 2012). However, the rearing of queen bees from *Apis cerana javana* from Indonesia has not been

studied. Therefore, this study was aimed to determine the response of age grafted larvae based on the acceptance rate and morphometric characteristics of the queen bee (*Apis cerana javana*).

MATERIALS AND METHODS

Study area

The study was performed in Kembang Joyo Honeybee Farm (7°52'38"S 112°34'34"E), Donowarih Village, Malang District, East Java, Indonesia.

Procedures

Grafting larvae

Grafting larvae of the bee *Apis cerana javana* was performed according to the previous method was conducted by De Souza et al. (2015). Briefly, seven colonies of *Apis cerana javana* with the same in productivity (queen bee from each colony was 1 year old) were used to take the larvae. The queen cells (top diameter was 0.71 cm, the bottom diameter was 0.62 cm, and height was 1 cm) have been stuck in the frame was used in the study was shown in Figure 1 and the frame was acclimatized in the hive for 24 hours in the hive. Afterward, all the queen cells were smeared using honey to stick the larvae in the queen cell base. Seventy-two of larvae (1, 2, and 3 days old where 24 larvae for each age) were taken using a grafting tool, then put into queen cells and divided into 6 colonies (queen bee was removed 8 to 9 days before of grafting process). Furthermore, all the frames were placed in the central colonies in the hive for 2 days to be fed royal jelly by the bee workers. In the third day, the frame was observed to check other queen cells made by the worker bees, and they were removed to the worker bees and focused on the frame used in the study. On

the fifth day, the queen cell acceptance was counted, characterized by the queen cell being covered by the wax, while the queen cell was not acceptance was still opened. The queen cells were hatches after 12 days of grafting and to protect the young queen bee from workers against, the queen cells were covered by queen cages.

Morphometric characteristics

The measurement of morphometric characteristics according to the previous method was reported by De Souza et al. (2015). After 12 days of grafting process, the hatches young queen was stored in an incubator CO₂ (temperature ranging from 34 to 35°C and humidity 70 to 80%) to faint the young queen. Afterward, the young queen bee was weighed by the digital scale and was measured the morphometrics characteristics using the combination of picture and Photoshop program version 22.4.2 (Adobe Inc.). The part of the queen bee which was measured was placed in a slide micrometer 0.01 mm, then an upright picture was taken and saved on the computer as an image. Afterward, the file was opened by the Photoshop program to measure the part of the queen bee was measured their morphometrics. The describe of the process to measure the morphometrics by Photoshop program is shown in Figure 2. The morphometrics characteristics were measured consisting of body length, width and length from the head, thorax, fore wing, and abdomen. All measurement was performed in 6 replicates, each in twice.

Data analysis

The morphometrics parameters data were analyzed by one-way analysis of variance using SPSS for Windows (Version 23.0) followed by Duncan's multiple range test and acceptance rate was analyzed by descriptive analysis.

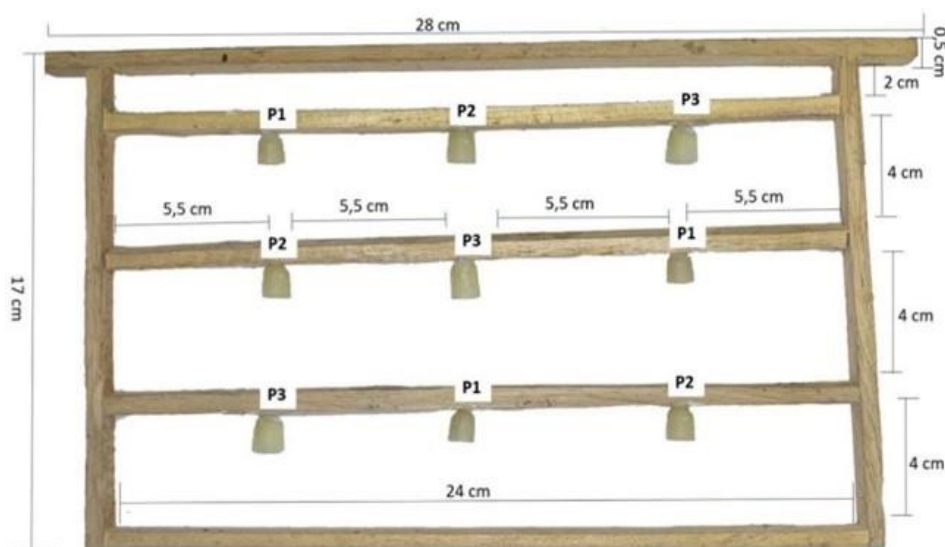


Figure 1. The frame and queen cell were used in the study

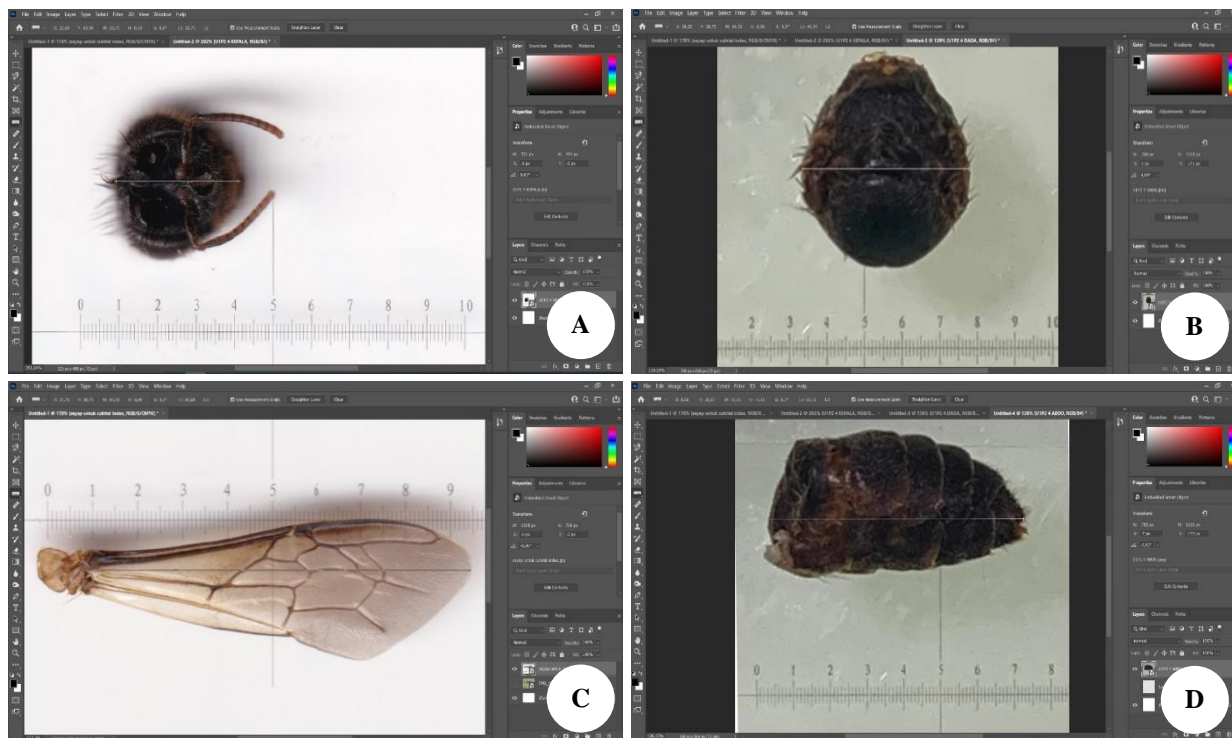


Figure 2. The photoshop display to measure the morphometrics characteristics of queen bee of *Apis cerana javana* (A. head; B. thorax; C. wing; D. abdomen)

RESULTS AND DISCUSSION

Results

The results showed that the acceptance rate of grafting larvae was higher in the 1-day old was 73.33% followed by 2 days old was 60%, and the lowest in 3 days old was 46.66% (Figure 3). The different ages for grafting larvae were increased the emergence weight and body length ($P < 0.01$), and abdomen length ($P < 0.05$), but not increased on the length and width of the head, thorax, fore wing, and abdomen length. The heavier of larvae emergence weight was found in 1 day old was 120.09 mg/queen, followed by 2 and 3 days old was 117.46 mg/queen and 115.99 mg/queen, respectively, however, the emergence weight among 2 and 3 days old did not differ. The body length of the queen bee was increased at 1 day old and was longer (13.02 mm) compared to 2 and 3 days old of larvae, 12.85 mm, and 12.87 mm, respectively ($P < 0.01$) however, among 2 and 3 days old were did not differ. The abdomen length in 1 day old larvae was higher (7.05 mm) than 2 and 3 days old, 6.99 mm and 6.85 mm, respectively, however, the abdomen length among 2 and 3 days old was similar (Table 1).

Discussion

The acceptance rate of grafting in 1 day old was affected by the queen cells, which was contained the young larvae are more visited by the worker bees to feed larvae using royal jelly than old larvae (Sagili et al. 2018). This finding also was similarly reported by Arun (2011) for *Apis mellifera* that the highest acceptance rate was found in 1

day old of larvae. However, was differed from reported by Cengiz et al. (2019) for *Apis mellifera* was found in 2 days old more which was acceptable compared to 1 day old. Furthermore, also was differ with reported by Okuyan and Akyol (2018) that the best acceptance rate (85.15%) for the rearing queen bee of *Apis mellifera anadolica* was found in 2 days old of larvae, however among 1, 2, and 3 days old of larvae did not differ on the acceptance rate. In addition, Njeru et al. (2017) was reported that the larvae age at 12, 24, and 36 hours was higher of acceptance rate compared to 6 and 48 hours for *Apis mellifera scutellata* queen. The acceptance rate of larvae was affected by the raw material to make queen cells, food, and seasons (Khan et al. 2021) and by honeybee species (Hussain et al. 2020).

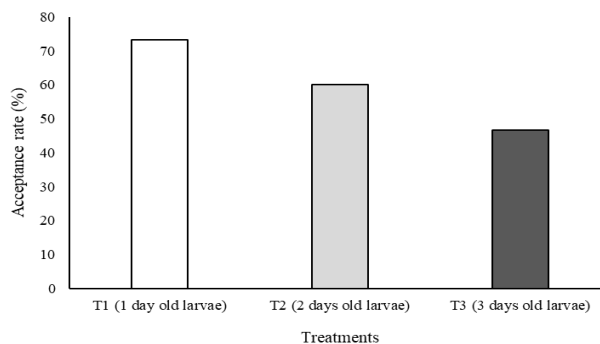


Figure 3. Acceptance rate (%) from grafting larvae at different ages of *Apis cerana javana*

Table 1. Morphometric characteristics of the queen bees by age of the grafted larvae

Morphometric characteristics	One day old	Two days old	Three days old
Emergence weight (mg)**	120.09± 0.91 ^b	117.46±1.21 ^a	115.99± 0.49 ^a
Body length (mm)**	13.02± 0.01 ^b	12.85± 0.08 ^a	12.87± 0.12 ^a
Head length (mm) ^{ns}	3.18±0.09	3.17±0.11	3.16±0.10
Head width (mm) ^{ns}	3.16±0.08	3.16±0.09	3.15±0.07
Thorax length (mm) ^{ns}	4.76 ±0.09	4.72±0.12	4.74±0.09
Thorax width(mm) ^{ns}	4.22±0.06	4.20±0.08	4.19±0.06
Fore wing length (mm) ^{ns}	2.85±0.11	3.84±0.09	3.83±0.08
Fore wing width (mm) ^{ns}	6.22±0.11	6.20±0.21	6.21±0.26
Abdomen length (mm)*	7.05± 0.08 ^b	6.99± 0.16 ^a	6.85± 0.13 ^a
Abdomen width (mm) ^{ns}	3.71±0.08	3.70±0.09	3.69±0.011

Note: **Significant at $P<0.01$; *Significant at $P<0.05$; ns: Not significant

The heavier emergence weight of 1 day old was supported by the higher acceptance rate in 1 day old than in 2 and 3 days old (Figure 3). In addition, the young larvae like 1 day old were more fed royal jelly by the worker bees than in old larvae such as 2 and 3 days old, because the young larvae were required a long time to feed of royal jelly than old larvae were a short time which was impacted on the bodyweight was heavier (Kuntadi 2013). This finding was similar with reported by Kuntadi (2013) reported that the queen bee of *Apis cerana*, which was produced by 1 day old larvae using a grafting technique, has a heavier body weight and higher egg production than higher 2 days old larvae. Furthermore, it was also similar to the report by Mahbobi et al. (2012), which showed that the queen bees reared from 1 day old of larvae had a significantly heavier bodyweight 158.83 mg/queen than 2 days old was 150.94 mg/queen. The queen bee size was directly related to the mating frequency, spermatheca size, ovum number, and ovary weight (Delaney et al. 2011; Tarpy et al. 2011). The morphometric characteristics of the queen bee was affected by the feeding supplementation (Njeru et al. 2011; Mahbobi et al. 2012), and the diameter of queen cells, which was related to the capacity of royal jelly, can be stored for queen bee cells (Wu et al. 2018). In addition, it was affected by macro and micro minerals (Alkahtani and Taha 2020) and the concentration of amino acids present in royal jelly (Rangel et al. 2016). The emergence weight of the young queen bee in our study was different with reported by Okuyan and Akhyol (2018) that the weight of the emerged queen bee of *Apis mellifera* from 1 day old ranged from 173.59 to 206.13 mg/queen.

The body length was longer from 1 day old was supported by the higher of acceptance rate and emerged weight of young queen bee in 1 day old compared to 2 and 3 days old (Figure 3) which was impacted on the much more of the royal jelly can be fed by the workers' bee than in 2 and 3 days old. The larvae age was a dominant factor that determined the quantity or amount of the royal jelly that workers bees can feed to larvae in queen cell, where the young larvae were obtained a royal jelly for a long time than in old larvae for a short time (Kuntadi 2013). This finding was similarly reported by Okuyan and Akhyol (2018) for *Apis mellifera anadolica* that the different age was a significant effect on the queen weight, body length,

thorax width and length, and wing length of a queen, but not on head width, head length, wing width and an acceptance rate of larvae. Furthermore, Mahbobi et al. (2012) also was found that 1-day old larvae for grafting have resulted from the young queen was larger body size compared to 2 and 3 days old. The body length of the young queen bee in our study was shorter compared to previously was reported by Okuyan and Akhyol (2018) was 16.72 mm for *Apis mellifera anadolica* and Arun (2011) was 14.99 mm for *Apis mellifera*.

The length and width of the head of young queen *Apis cerana javana* in our study did not differ among the treatments, however, the emergence weight of young queen bee did differ. Okuyan and Akhyol (2018) reported that the head's length and width of *Apis mellifera anadolica* have the lowest correlation with young queen weight compared to body length as the highest correlation with the young queen weight. This finding differed from the report by Mahbobi that 1 day old was increased the length of the head of young queen from *Apis mellifera* than 2 days old. The head's length and width in our study differed to reported by Okuyan and Akhyol (2018) for *Apis mellifera anadolica* ranging from 3.49 to 3.56 mm and 3.82 to 3.88 mm, respectively. Furthermore, ranging 1.78 to 1.88 mm for the head length and 1.81 to 1.97 mm for the head width from the *Apis mellifera scutellata* queen from different of larvae age (6, 12, 24, and 36 hours) (Njeru et al. 2017).

The width and length of the thorax of young queen *Apis cerana javana* in our study did not differ among the treatments. However, Tarpy et al. (2011) was reported that the thorax of queen bee was reared from 1 day old was wider than 2 days old. The different results were affected by the genetic of bee species and environmental conditions (Mahbobi et al. 2012). The width and length of the thorax of queen bee in our study were differed to reported by Njeru et al. (2017) that the width and length of thorax from *Apis mellifera scutellata* queen (reared from a different age: 6, 12, 24, and 36 hours old) ranging from 1.57 to 1.65 mm and 4.79 to 4.97 mm, respectively. Furthermore, it also was differed to reported by Okuyan and Akhyol (2018) was 4.43 mm for width and 4.07 mm for a length of a young queen from *Apis mellifera anadolica*.

The width and length of the fore wings of young queen *Apis cerana javana* in our study did not differ among the

treatments. This finding was differed to reported by Mahbobi et al. (2012) that the larvae age had a significant effect on the fore wing width of *Apis mellifera meda*. The width and length of the fore wings of young queen *Apis cerana javana* in our study differed to those reported by Okuyan and Akyol (2018) that the width and length of the young queen of *Apis mellifera anadolica* ranging 3.10 to 3.14 mm and 10.34 to 10.55 mm, respectively. The abdomen length of the young queen from *Apis cerana javana* was higher in 1 day old larvae compared to 2 and 3 days old larvae was supported by the higher acceptance rate, heavier emergence weight, and longer body length. This finding was similar with reported by Minarti and Akhroh (2021) that the abdomen of *Apis mellifera* queen was grafted from 1 day old larvae that were longer than 2 and 3 days old larvae. Vung et al. (2018) reported that the queen bee's high quality from *Apis cerana* colonies was produced after 270 days of the established colonies, namely 90 days. Thus, overall, of the morphometric characteristics were affected by the availability of royal jelly in queen cells (related to the availability of food, especially pollen), the bee species, queen cell size, the population of workers bees, and environmental condition (temperature, humidity, and climatic) (Njeru et al. 2017). Thus, it can be concluded that the different age of grafting *Apis cerana javana* influences the morphometric characteristics. The 1 day old larvae grafting *Apis cerana javana* has improved the acceptance rate, emergence weight, body, and abdomen length compared to 2 and 3 days old larvae, however, it has not improved the other morphometric characteristics.

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