BIODIVERSITAS ISSN: 1412-033X
Volume 24, Number 10, October 2023 E-ISSN: 2085-4722
Pages: 5626-5633 DOI: 10.13057/biodiv/d241044

Evaluation of body weight and morphometric characteristics of the young queen of *Apis cerana javana* at different queen cup cell sizes

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Manuscript received: 23 July 2023. Revision accepted: 26 October 2023.

Abstract. *Masyithoh D, Kentjonowaty I, Salleh SM, Ustadi, Agussalim, Nastain FS. 2023. Evaluation of body weight and morphometric characteristics of the young queen of* Apis cerana javana *at different queen cup cell sizes. Biodiversitas 24: 5626-5633.* This study investigates body weight and morphometric characteristics of *Apis cerana javana* queen rearing based on differences in the queen cup sizes. The queen cell sizes were divided into three categories; the first group was a small category (T1) with a height of 0.75 cm, top diameter of 0.65 cm, and bottom diameter of 0.44 cm. The second treatment was medium category (T2) with a height of 0.85 cm, a top diameter of 0.71 cm, and a bottom diameter of 0.54 cm. The third treatment was a large category (T3) with a height of 1.00 cm, a top diameter of 0.71 cm, and a bottom diameter of 0.62 cm. The morphometrics investigated consisted of hatch weight, pupae length, body length, head, abdomen, wing, and hind leg morphometrics. The results showed that the differences in the queen cup size had no effect (P>0.05) on the hatch weight, body length, width, and length of the head, thorax, fore and hind wing (length and width), and abdomen (length and height). The different queen cell sizes did not affect the wing morphometrics (P>0.05). In addition, the leg morphometrics consisting of femur, tibia, tarsus, and metatarsus were also not affected (P>0.05) by the different queen cup sizes. These results revealed that the different queen cup sizes did not negatively impact the morphometric characteristics of *A. cerana javana* queen's.

Keywords: *Apis cerana*, morphometrics, queen bee, queen cup size

INTRODUCTION

Indonesia has the widest diversity of honey bees (*Apis* spp.) in the world (Kahono 2018). *Apis cerana* Fabricius, 1793, is spread in almost all the islands in Indonesia, up to Timor, except in Maluku and Irian. According to several sources, *A. cerana* in Ambon and Irian are not native to the island but are imported from other areas (Hadisoesilo and Kahono 2011). *Apis cerana* shows greater hygienic behavior, making it more disease-resistant and enabling it to coexist with Varroa mites (Koetz 2013). There are several advantages of *Apis cerana* as an efficient pollinator; the first is a small pollinator, which means that each worker spends more time with the same plants and has higher floral constancy. The smaller colony size of *A. cerana* is also encouraging, as it makes them easy to transport and manage. Furthermore, in some conditions, *A. cerana* is considered a superior pollinator compared to *Apis mellifera* (Egelie et al. 2015).

Successful honey bee production and profitable beekeeping depend greatly on the quality of honey bee queens (Büchler et al. 2013). The state of a colony is significantly influenced by the queen's productivity and health (Fine et al. 2018). Queen rearing is the most crucial beekeeping technique, which helps honeybee colonies grow quickly. It also allows beekeepers to introduce new queens in case of sudden loss during colony manipulation, transportation, or due to the attack of enemies or diseases that affect honeybees (Sharma et al. 2020).

Numerous researchers have documented how colonies react to various queen-rearing methods due to environmental, behavioral, and biological variations (Crailsheim et al. 2013). Temperature, relative humidity, and pollen availability are important climate variables influencing whether artificially produced queens are accepted and high-quality (Jagdale et al. 2021). The physical characteristics of a queen, such as weight, thorax and head width, number of ovarioles, spermathecal size, and number of stored spermatozoa, are used to assess its quality. Breed, age, and the quantity of grafted larvae, as well as the rearing season and feeding, are environmental and genetic factors that influence the quality of the queen (Haldhar et al. 2021a, 2021b; Büchler et al. 2013).

The size of the queen cell cup may have an impact on the acceptance rate of grafted larvae as well as the size of the queen, both of which may have an impact on the quality of the colony. The weights of the queens' body parts (head weight, thorax width and weight, abdomen width and weight) appear to be significantly influenced by the size of the cell cup (Mattiello et al. 2022). For instance, the size of the cell cup where queen larvae are raised may impact the fitness and capacity for the reproduction of the young queens (Wu et al. 2018). According to Büchler et al.

(2013), the ideal queen cell cup size is between 8.0 and 9.0 mm in diameter at the rim. Adgaba et al. (2019) did not discover any differences in the acceptance rate of grafted larvae in *Apis mellifera jemenitica* using smaller cell cup sizes (7.0 mm, 7.5 mm, 8.0 mm, and 8.5 mm). On the other hand, body weight, thorax length, and thorax width of *Apis mellifera ligustica* queens at emergence were significantly impacted by different cell cup sizes (9.4 mm, 9.6 mm, 9.8 mm, and 10.0 mm) (Wu et al. 2018). However, the size of a cell has been shown to affect queen-worker differentiation, but a cell could be 9.6, 9.8, or 10.0 mm in size (Shi et al. 2011). In addition, the morphometric characteristics of young queen *A. cerana javana* at different queen cell sizes have yet to be studied. Moreover, appropriate queen cell size will produce good quality morphometric characteristics. The current study aims to investigate the effect of different queen cell sizes on the body weight and morphometrics of *A. cerana javana* queen rearing.

MATERIALS AND METHODS

Study area

This study was performed at Kembang Joyo farm for beekeeping of *A. cerana javana* (7°52'45°S 112°34'54''E) in Donowarih Village, Karangploso Sub-district, Malang District. Seven colonies of *A. cerana javana* each contained five frames completed by honey and bee bread for the bee's consumption. All colonies were used as the larvae source for grafting queen cells, whereas the larva was used at 1 day old.

Study design

In this study, we used three treatments of queen cell sizes and six replications (every replication has three queen cells). The queen cell sizes were divided into three categories; the first group was a small category (T1) with a height of 0.75 cm, top diameter of 0.65 cm, and bottom diameter of 0.44 cm. The second treatment was the medium category (T2), with a height of 0.85 cm, a top diameter of 0.71 cm, and a bottom diameter of 0.54 cm. The third treatment was a large category (T3) with a height of 1.00 cm, a top diameter of 0.71 cm, and a bottom diameter of 0.62 cm. The Molded wood was used to create a queen cell, as shown in Figure 1.

Procedures

Queen cell cups

The comb from *A. cerana javana* was boiled using water, then filtered, and deposited one night after melting. Afterward, the wax in the top was taken and melted in the double jacketed water bath at 65°C, just above the melting point. The wood ends were dipped into cold water and then into wax liquid up to marker for 3 to 5 seconds (Figure 2A). Furthermore, the queen cell cups were removed and dipped into cold water. Queen cell cups were bowled, ready for grafting larvae to make *A. cerana javana* queen (Figure 2B).

Placing queen cell cups in the frame

The queen cell cups were randomly positioned and attached to the frame using melted wax. The height frame was 17 cm, the width was 24 cm, the length was 28 cm, and the distance among queen cell cups was 5.5 cm, shown in Figure 3. Furthermore, this frame was put into the center of the *A. cerana javana* colony to introduce the frame to honeybee workers before larvae were grafted.

Larvae grafting

Grafting the *A. cerana javana* larvae was done according to the method described by Ustadi et al. (2022a). The frame contains queen cell cups adapted to the colony and placed in the center for 24 hours. The queen bee colony was removed 8 to 9 days before grafting larvae. Next, 72 larvae were taken from the colony one day using a grafting tool and put into queen cell cups stuck by honey to stick larvae in the base. Afterward, the frame was left for 2 days to be fed royal jelly by worker bees, and on the 3rd day, the frame was checked to remove another queen cell made by worker bees so worker bees could focus on carrying the queen bee candidates. The queen cell was accepted by workers bee characterized by queen cell cups that had been covered (on the 5th day) by thin wax, but when rejected, the queen cell was still opened. On the 5th day, queen bee candidates had become the pupae stage and a digital vernier caliper measured body length. Afterward, the queen cell cups containing pupae were moved to the queen cell cage to protect them from damage and make them easier to take when hatched. On the 12th day, the queen bee was hatched in the queen cage, and the new queen was put into an incubator with CO_2 (20%) at a temperature of 34 to 35°C and humidity of 70 to 80%.

Figure 1. The equipment to create queen cell cups of various sizes (small, medium, and large)

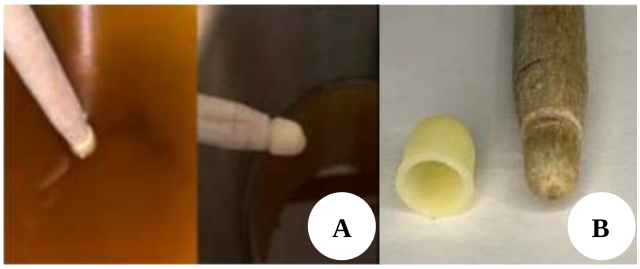

Figure 2. The process to make queen cell cups of various sizes (small, medium, and large)

Morphometric parameters

After the young queen was hatched, they fainted using CO_2 gas easily when cell length and morphometric characteristics were measured. Morphometrics characteristics were measured by combining picture and Photoshop programs (version 22.4.2, Adobe Inc.) according to the method described by Ustadi et al. (2022a). Briefly, the part of the queen was measured in microscope objective micrometer-slide glass (0.01 mm). Afterward, the picture of the queen bee was taken and saved on the computer as an image, then opened by the Photoshop program to measure their morphometric parts. The description of the process of measuring the morphometrics by the Photoshop program is shown in Figure 4. The morphometrics characteristics from the young queen of *A. cerana javana* were measured consisting of body length, width and length from the head, thorax, and fore and hind wing (length and width), hatched weight, pupae length, and length and height of the abdomen, respectively. Furthermore, the wing morphometrics were studied consisting of cubital or length (A and B), cubital index (A/B), length (C and D), radial field, dumb-bell index, inner wing (length and width), and radial cell or length I, II, III, IV, respectively (Figure 4). Leg morphometrics consisted of femur, tibia, tarsus, and metatarsus. The wet weight of the young queen of *A. cerana javana* was weighed by a digital scale with an accuracy of 0.01 g. All measurement was performed in 6 replicates (every replicates have three queen cells) and measured twice.

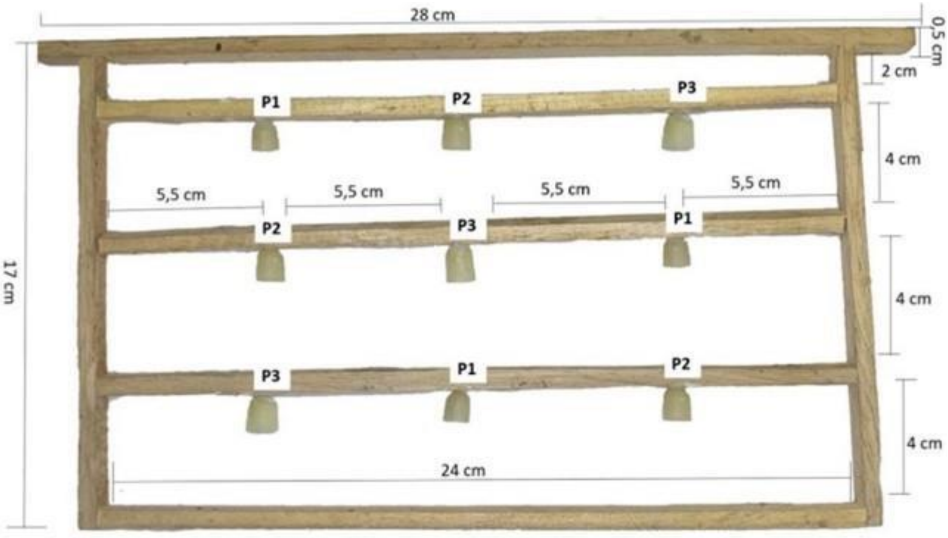

Figure 3. Frame used to stick queen cell cups (P1: small (T1), P2: medium (T2), and P3: large (T3)) from *A. cerana javana* larvae

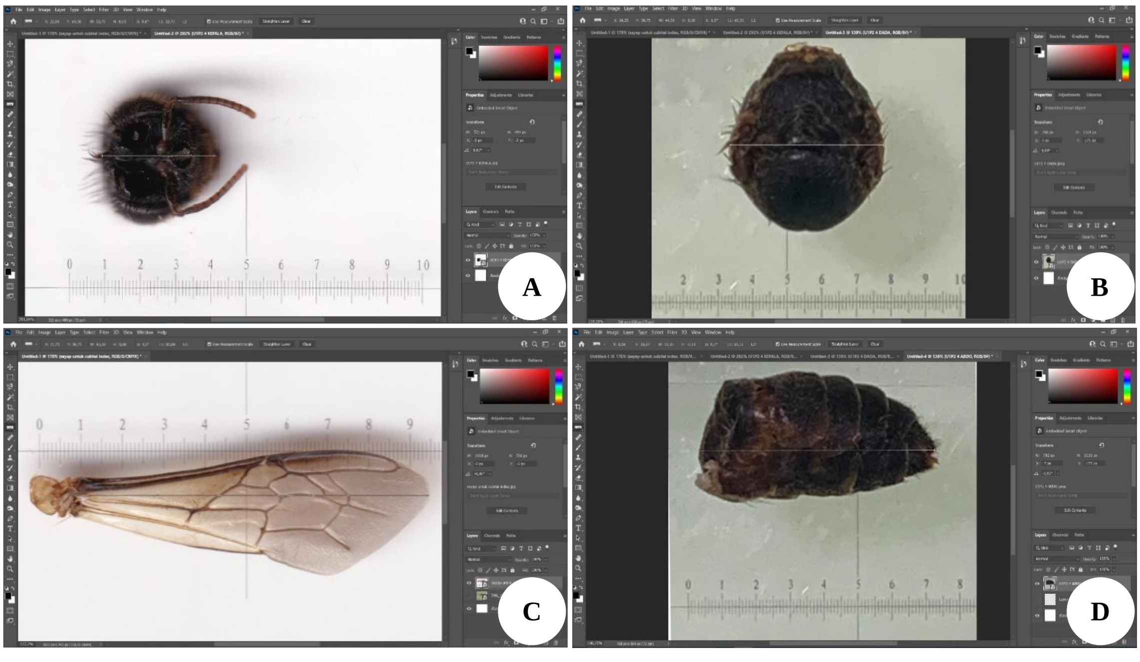

Figure 4. The photoshop display was used to measure the morphometric characteristics of *A. cerana javana* queen (A. Head, B. Thorax, C. Wing, D. Abdomen) (Ustadi et al. 2022a)

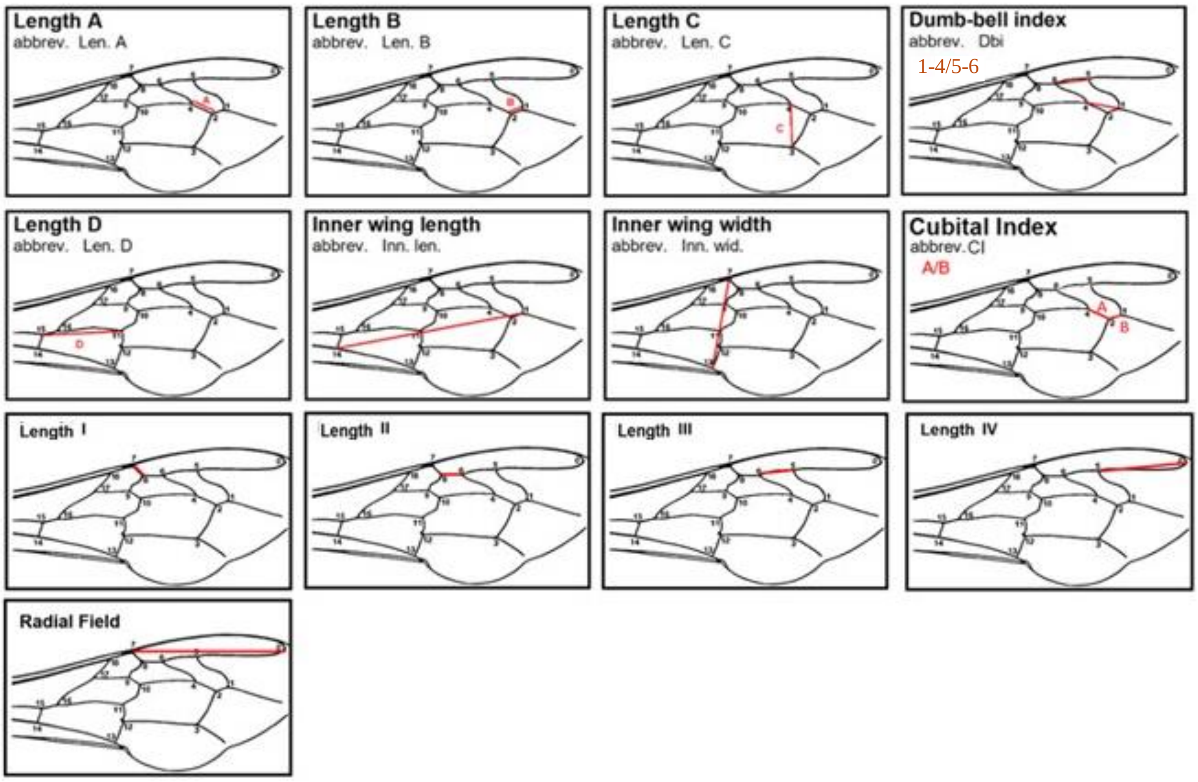

Figure 5. The guidelines to measure wing morphometrics from the honeybee (Bouga et al. 2011)

Data analysis

The morphometric characteristics of different cell cup sizes were analyzed using one-way variance analysis with a significant level of p<0.05.

RESULTS AND DISCUSSION

Results

The recent results showed that the different queen cell sizes have no significant effect (P>0.05) on the hatches weight, body length, width, and length of the head, thorax, fore and hind wing (length and width), and abdomen (length and height), respectively (Table 1). Furthermore, the wing morphometrics of cubital A and B, cubital index (A/B), length C and D, radial field, dumb-bell index, inner wing (length and width), and radial cell or length I, II, III, and IV were not affected (P>0.05) by the different queen cell sizes (Table 2). In addition, the leg morphometrics consisting of femur, tibia, tarsus, and metatarsus were also not affected (P>0.05) by the different queen cell sizes (Table 3).

Based on the data in Table 1, the results of the variance analysis showed that the difference in queen cup size did not have a significant effect on the morphometric characteristics of queen bees (*A. cerana javana*) (P>0.05).

Based on the diversity analysis of the data (Table 2), it is shown that the differences in queen cup size did not affect the morphometric characteristics of queen bee wings (P>0.05).

Discussion

The information on queen cell sizes used to graft queen larvae is crucial for beekeepers, especially in Indonesia, to better develop *A. cerana* generally. The morphometric characteristics of the *A. cerana javana* young queen bee in our study were unaffected by the different queen cell sizes. These results indicate that the *A. cerana javana* worker has been modified in the queen cell, which impacts the similar size. Therefore, maybe the nurse bees are fed royal jelly in equal quantity; however, in our study, it is not measured.

Table 1. Morphometric characteristics from the young queen of *A. cerana javana* at different queen cell sizes

Morphometric characteristics	Queen cell sizes		
	Small	Medium	Large
Hatches weight (mg)	112.33±0.17	112.78±12.06	113.93±1.53
Cell length (mm)	16.57±0.42	16.66±0.62	16.74±0.75
Body length (mm)	9.30±0.50	9.66±0.88	9.75±0.71
Head width (mm)	3.17±0.02	3.19±0.03	3.18±0.0
Head length (mm)	2.89±0.07	3.19±0.09	2.95±0.05
Thorax width (mm)	3.15±0.04	3.18±0.02	3.22±0.17
Thorax length (mm)	3.10±0.03	3.13±0.07	3.15±0.02
Forewing width (mm)	3.65±0.04	3.68±0.03	3.70±0.05
Forewing length (mm)	8.37±0.08	8.39±0.07	8.42±0.17
Hind wing width (mm)	1.81±0.02	1.82±0.02	1.84±0.04
Hind wing length (mm)	6.18±0.02	6.20±0.02	6.21±0.04
Abdomen length (mm)	5.19±0.04	5.21±0.14	5.28±0.17
Abdomen height (mm)	3.65±0.04	3.68±0.03	3.70±0.05

Note: Data were presented in mean ± standard deviation

Table 2. Wing morphometrics from the young queen of *A. cerana javana* at different queen cell sizes

Morphometric characteristics	Queen cell sizes		
	Small	Medium	Large
Cubital A (mm)	0.41±0.02	0.42±0.01	0.42±0.01
Cubital B (mm)	0.13±0.01	0.14±0.02	0.14±0.01
Cubital index	3.14±0.32	3.00±0.43	3.09±0.30
Length C (mm)	0.77±0.03	0.79±0.03	0.81±0.30
Length D (mm)	1.81±0.02	1.82±0.02	1.84±0.04
Dumb-bell index	0.98±0.03	1.00±0.03	1.00±0.02
Inner wing length (mm)	3.96±0.03	3.97±0.02	4.00±0.03
Inner wing width (mm)	2.01±0.03	2.00±0.03	2.02±0.02
Radial field (mm)	2.85±0.02	2.86±0.02	2.87±0.01
Radial cell I (mm)	0.31±0.02	0.33±0.06	0.33±0.01
Radial cell II (mm)	0.41±0.01	0.42±0.02	0.44±0.03
Radial cell III (mm)	0.60±0.02	0.59±0.01	0.62±0.03
Radial cell IV (mm)	1.62±0.02	1.63±0.01	1.63±0.08

Note: Data were presented in mean ± standard deviation

Table 3. Hind leg morphometrics from the young queen of *A. cerana javana* at different queen cell sizes

Morphometric characteristics	Queen cell sizes		
	Small	Medium	Large
Femur (mm)	2.45±0.04	2.47±0.07	2.49±0.03
Tibia (mm)	2.66±0.04	2.66±0.07	2.68±0.01
Metatarsus (mm)	1.70±0.02	1.72±0.06	1.72±0.02
Tarsus (mm)	1.40±0.02	1.49±0.06	1.50±0.03

Note: Data were presented in mean ± standard deviation

In terms of wet weight (hatches weight) morphometry, an increase is directly proportional to the queen cup size. Larger cup sizes result in queen bees with higher wet weights than smaller cup sizes, with a difference of 1.6 mg. However, statistically, this difference is not significant. Wet weight is considered one of the most important morphological characteristics related to the quality of queen bees (Amiri et al. 2017). According to Novita et al. (2013), the quantity and quality of the food provided by worker bees significantly influence the development of potential queen bees, closely related to morphological characteristics. The cup cell sizes of queen bees used in the study were obtained directly from queen cells of various original sizes. According to Mattiello et al. (2022), queen cell sizes that are close to the original size yield the best results compared to larger or smaller sizes.

Wu et al. (2018) state that the increase in wet weight is directly proportional to the diameter of the queen cup used, and wet weight significantly increases when a larger-diameter queen cup is used. This is because larger queen bee cups have more space, which promotes the development of potential queen bees and increases the wet weight of the queen. Wu et al. (2018) also explain that the height of royal jelly in queen bee cells of various sizes maintained simultaneously yields the same height. Consequently, larvae reared in cells with wider diameters receive more royal jelly, and vice versa. Wet weight can be used to estimate queen bees' quality because queen bees with heavier weights have more ovarioles, larger spermathecae, and more spermatozoa in the spermatheca (Arslan et al. 2021). Kamyab et al. (2020) explain that queen bees with higher body weights have better acceptance and increase their successful introduction by recipient colonies than queen bees with lower body weights. Mattiello et al. (2022) also emphasize that the wet weight of queen bees can be used as a selection criterion.

Based on assumption tests and variance analysis, it can be concluded that the length of pupae in the queen bee *A. cerana javana* is not influenced by the size of the cups used in this study. These findings differ from those of Mubarok et al. (2020) regarding the influence of queen cell size on *Apis mellifera*, which stated that smaller queen cell diameters result in longer pupae. The lack of significant difference in pupal length could be attributed to the fact that the queen cell size was modified by worker bees, resulting in a more uniform provision of royal jelly by the worker bees. As a result, the length of potential queen bee pupae becomes more consistent. Wu et al. (2018) explained that the height of royal jelly in queen bee cells of various sizes, maintained simultaneously, resulted in the same height. The pupal stage is the most prolonged phase compared to the egg and larval stages. The pupal phase in bee larvae lasts 6 to 7.5 days inside the cell (Kuntadi 2013). During the pupal phase, bee larvae change body shape, and their organs develop. Organ formation begins during the pupal stage, including the eyes, legs, and wings (Kaleka et al. 2019).

Then, the characteristic of morphometric body length of the candidate queen bee *A. cerana javana* is known that the worker bees of *A. cerana javana* tend to build uniform pupae in all types of queen cell sizes (Table 1), indicating that the food received by the larvae tends to be the same, thus not resulting in differences in body length and wet weight of the candidate queen bee. Kuntadi (2013) explained that providing royal jelly depends on the shape and size of the cell where the larvae develop. As for the length and width of the head of the candidate queen bee *A. cerana javana*, it has a smaller average head width compared to the findings of Ibrahim et al. (2019), who studied the morphometry of *Apis cerana indica* honey bees in Pradesh India, with an average head width of queen bees ranging from 3.49 to 3.78 mm. This is likely due to genotype differences in the bees used for research. In addition, the quality of the queens being raised is influenced by many factors, such as the season of rearing, queen breeding methods, and the age of the larvae used for queen rearing (Hatjina et al. 2014; He et al. 2017). The average head length in this study is still within the normal range, consistent with the study by Ibrahim et al. (2019), where the average head length of *Apis cerana* queen bees was a minimum of 2.87 mm. The similar results in the morphometric measurements of head length and width could be attributed to the similarity in the size of the pupae.

Next, the research results on the morphometric characteristics of the length and width of the thorax of the *A. cerana javana* queen bee differ from the findings of Wu et al. (2018) that the height and width of the thorax of *Apis mellifera* L. significantly increased when using larger-sized queen cups. The different results could be attributed to genotype differences and environmental factors. Wu et al. (2018) conducted the research in China using *Apis mellifera* L. bees, while this study was conducted in Indonesia. Different food sources and seasons can influence the growth and development of the candidate queen bee larvae. According to Kamyab et al. (2020), the nutritional content of royal jelly obtained from different seasons shows different nutrient compositions. Additionally, according to Alburaki et al. (2017), the cumulative weight gain of colony members is influenced by the initial weight of colony members at the beginning of the flowering season. The research by Delaney et al. (2011) reported that queens with more prominent thorax characteristics tend to mate with a more significant number of drones, and a positive correlation was found between thorax width, mating frequency, and the amount of sperm that can be stored (averaging 3.99 million sperm obtained from 115 queens).

The morphometric characteristics of the length and width of the forewing and hindwing of the queen bee *A. cerana javana* are not influenced by the size of small, medium, and large cups. Food is essential to larval growth (Pang et al. 2022). The consistent results in wing length and width may be due to the tendency of *A. cerana javana* worker bees to provide a uniform quantity of royal jelly as food (Minarti et al. 2022). The length of the queen bee's forewing is taken from the wing base to the outermost part of the forewing, and the width is taken at the widest part of the forewing. The results of the measurement of the length and width of the forewing differ from the study by Novita et al. (2013), where the forewing length of *Apis cerana* is 7.42 mm and the forewing width is 2.60 mm. The survey by Mahbobi et al. (2012) also found significant differences in wing length among different ages of grafted larvae. Based on the findings of these researchers, it can be concluded that genotype differences and the age of grafted larvae can also affect wing morphometric measurements. The queen bee's wings serve as the body organ for flying during mating with drones and support high productivity for colony survival. Not only is there a difference in wing length, but there is also a difference in wing width between worker bees and queen bees of *Apis cerana*. The average width of worker bee wings is 2.57 to 2.60 mm, smaller than the queen bee's (Novita et al. 2013).

The length and height of the abdomen in morphometric measurements of the queen bee *A. cerana javana* are unaffected by queen cup size differences. This may be due to the use of larvae of the same age in the study, which means that the time for completing the larval stage to pupa is the same. This is different from the study by Wu et al. (2018), which explained that the size of the queen cup diameter results in a larger intake of royal jelly by the queen bee *Apis mellifera* L. The results of this study differ from Wu et al. (2018), possibly due to the different bee species and environmental differences during the experimental period, leading to different responses received by the larvae. The study by Metorima et al. (2015) used abdomen size as a morphometric characteristic for selecting potential royal jelly producers as new queen bees. Queens with larger abdomens have a high potential for success in artificial insemination. The queen bee's abdomen-size characteristics differ from those of worker bees and drones. The queen bee's abdomen is more extended. This can be associated with the queen bee's role in egg production within a colony. According to Baer et al. (2016), the size of the queen bee's abdomen affects the amount of sperm that can be stored in the spermatheca, and the larger the abdomen, the greater the egg production. This is consistent with Rangel et al. (2016), who reported that the queen bee's egg-laying ability is based on the capacity of the ovaries and the volume of the spermatheca in its reproductive organs, and the volume of the ovaries is directly proportional to the number of egg tubes (ovarioles). Productive queen bees are characterized by large reproductive organ capacity.

Based on the homogeneity test and the additivity test of queen cup size data against cubital morphometry, cubital index, length, dumb-bell index, inner wing, radial field, as well as *radial cells* I, II, III, and IV of the queen bee's wings (*A. cerana javana*), the data exhibited homogeneity and additivity characteristics. The lack of significant differences may be due to the queen bee strains' similarity in this study. This is consistent with Hassona (2017) explanation that the Cubital section of the bee wing is a specific part unique to each population of honey bees. The Cubital section of the bee wing consists of two parts, A and B. The measurements of Cubital A and B differ from the findings of Dolasevic et al. (2020) on *Apis mellifera* queen bees. Cubital A is 0.53 to 0.54 mm, longer than *A. cerana javana* queen bees in our study (Table 2). Cubital B in *Apis mellifera* queen bees has a length of 0.22 to 0.23 mm, which is longer than that of *A. cerana javana* queen bees in our study (Table 2). The Cubital Index in morphometrics is the ratio between the two wing vein segments of honey bees, specifically the ratio between cubital A and cubital B (Hassona 2017). The Cubital Index is calculated by dividing cubital A by cubital B, i.e., Cubital Index (A/B). However, the Cubital Index from a small size of queen cup size is larger than that of large and medium sizes. The mean value of this study is smaller than the findings of Li et al. (2020) where the mean Cubital Index value of *Apis cerana nuluensis* is 3.84 in Sulawesi, indicating that genotype and environment differences influence the size of the wing's Cubital Index. Frunze et al. (2021) explained that the cubital index characteristic is used to distinguish subspecies of honey bees. Based on the Ustadi et al. (2022b), research shows their findings on the differences in Cubital Index values between *Apis cerana* and *Apis florea queen* bees. It can be concluded that genotype and regional differences influence the value of the Cubital Index (Hassona 2017).

Next, the morphometric lengths C and D in this study for the queen bees of *A. cerana javana* are not different from the queen bees of *Apis mellifera* in the study by Dolasevic et al. (2020) where the size of length C in *Apis mellifera* queen bees ranges from 0.77 to 0.81 mm, while the size of length D is different, ranging from 1.93 to 2.00 mm. The morphometric dumb-bell index (1-4/5-6), indicates that the larger the size of the hive, the higher the dumb-bell index value of the queen bees of *A. cerana* *javana*. However, although there is a positive correlation graphically, the hive size does not influence the dumb-bell index (1-4/5-6) (range 0.98 to 1.00, Table 2). This may be because the queen bees used in the study are of the same strain, *A. cerana javana*; thus, there is no significant difference in the morphometric dumb-bell index values of the resulting queen bees (Ji et al. 2023). In morphometry, the inner wing length will show differences in different honeybee species. Ostwald et al. (2020) stated that the inner wing length morphometry of the carpenter bee (*Xylocopa confuse* Linn.) has a length of 12.8 to 13.59 mm for the hind wing and 19.64 to 20.56 mm for the forewing. The wing width also differs in size, with the hind wing measuring 4.43 to 4.75 mm and the forewing measuring 5.97 to 6.15 mm. The differences in size occur because measurements are carried out on different bee species. Morphometry is used to study honeybees' genetic diversity and the morphological characteristics of bees to determine the diversity of honeybee species (Bustamante et al. 2021).

Furthermore, the morphometry of the radial field (0-7) and radial cells I (7-8), II (6-8), III (5-8), and IV (0.5) were unaffected by the differences in queen cup size. The research conducted by Dolasevic et al. (2020) showed that the queen bees of *Apis mellifera* have different wing angles than those of *Apis cerana*. This strengthens the idea that honeybee morphometry can be used for species and subspecies identification of honeybees (Ropars et al. 2021).

The homogeneity test results and the additive effect of queen cup size on the femur, tibia, metatarsus, and tarsus morphometry were homogeneous and additive. The mean measurements of the morphometry of the hind legs are presented in Table 3. Based on the diversity analysis of the data (Table 3), it is shown that the morphometry of the femur, tibia, metatarsus, and tarsus of *A. cerana javana* queen bees in our study did not differ among the treatments. This means that an increase in queen cup size, which results in increased cell space, does not increase the size of the queen bee's hind legs. This differs from the findings of Wu et al. (2018), which showed that an increase in cell diameter improves the quality of queen bees. The similar results may be due to the intake of royal jelly from worker bees, which tends to be the same. The femur is a part of the bee's leg used as support during flight. The study by Novita et al. (2013) on the length of the femur of bees raised in the highland area of Kepahiang found that it was longer than those in the lowland area of Bengkulu, which may be attributed to environmental differences. Suhri et al. (2022) reported that flight activity is influenced by temperature, wind, and light intensity. Changes in the tibia's morphology and metatarsus are linked to daily activities, and these organ activities are not the primary factors but can be influenced by environmental factors such as temperature and air (Novita et al. 2013). Air temperature, humidity, and light intensity are closely related to food availability (Resti et al. 2022). The bee's leg terminates in the tarsus. The tarsus has two branched claws for gripping surfaces (Rocha et al. 2022). The study examines the influence of queen cup cell sizes on the morphometric characteristics of *A. cerana javana* queen bees. It reveals that queen cell sizes don't significantly affect most characteristics of young queen bees, including body length, head width and length, thorax width and length, wing width and length, and abdomen length and height. Despite a slight increase in wet weight in larger queen cup sizes, statistical significance is not observed. Factors such as worker bees' uniform provision of royal jelly and genetic similarity are possible causes of this homogeneity. Furthermore, genotype and regional differences are highlighted as significant influences on the morphometric characteristics of bees, especially in the Cubital Index values of queen bee wings. Lastly, it is concluded that queen cell size has no substantial influence on the morphometric characteristics of *A. cerana javana* queen bees and that other factors such as genotype, environment, and the quality and quantity of nutrition provided by worker bees are more influential in bee development.

ACKNOWLEDGEMENTS

The authors are grateful to PT Kembang Joyo Sriwijaya Malang for providing financial support.

REFERENCES

Adgaba N, Al-Ghamdi A, Tadesse Y, Alsarhan R, Single A, Mohammed SE, Ali Khan K. 2019. The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques. Saudi J Biol Sci 26 (7): 1649-1654. DOI: 10.1016/j.sjbs.2018.08.028.

Alburaki M, Steckel SJ, Williams MT, Skinner JA, Tarpy DR, Meikle WG, Adamczyk J, Stewart SD. 2017. Agricultural landscape and pesticide effects on honey bee (Hymenoptera: Apidae) biological traits. J Econ Entomol 110 (3): 835-847. DOI: 10.1093/jee/tox111.

Amiri E, Strand MK, Rueppell O, Tarpy DR. 2017. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. Insects 8 (2): 48. DOI: 10.3390/insects8020048.

Arslan S, Cengiz MM, Gül A, Sayed S. 2021. Evaluation of the standards compliance of the queen bees reared in the Mediterranean region in Turkey. Saudi J Biol Sci 28 (5): 2686-2691. DOI: 10.1016/j.sjbs.2021.03.009.

Baer B, Collins J, Maalaps K, den Boer SPA. 2016. Sperm use economy of honeybee (*Apis mellifera*) queens. Ecol Evol 6 (9): 2877-2885. DOI: 10.1002/ece3.2075.

Bouga M, Alaux C, Bienkowska M, Büchler R, Carreck NL, Cauia E, Chlebo R, Dahle B, Dall'Olio R, De La Rúa P, Gregorc A, Ivanova E, Kence A, Kence M, Kezic N, Kiprijanovska H, Kozmus P, Kryger P, Le Conte Y, Lodesani M, Murilhas AM, Siceanu A, Soland G, Uzunov A, Wilde J. 2011. A review of methods for discrimination of honey bee populations as applied to European beekeeping. J Apic Res 50 (1): 51-84. DOI: 10.3896/IBRA.1.50.1.06.

Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N, Kryger P, Spivak M, Uzunov A, Wilde J. 2013. Standard methods for rearing and selection of *Apis mellifera* queens. J Apic Res 52 (1): 1-30. DOI: 10.3896/IBRA.1.52.1.07.

Bustamante T, Fuchs S, Grünewald B, Ellis JD. 2021. A geometric morphometric method and web application for identifying honey bee species (*Apis* spp.) using only forewings. Apidologie 52 (3): 697-706. DOI: 10.1007/s13592-021-00857-7.

Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, Vollmann J, Riessberger-Gallé U. 2013. Standard methods for artificial rearing of *Apis mellifera* larvae. J Apic Res 52: 1-16. DOI: 10.3896/IBRA.1.52.1.05.

Delaney DA, Keller JJ, Caren JR, Tarpy DR. 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). Apidologie 42 (1): 1-13. DOI: 10.1051/apido/2010027.

Dolasevic S, Stevanovic J, Aleksic N, Glavinic U, Deletic N, Mladenovic M, Stanimirovic Z. 2020. The effect of diet types on some quality characteristics of artificially reared *Apis mellifera* queens. J Apic Res 59 (1): 115-123. DOI: 10.1080/00218839.2019.1673965.

Egelie AA, Mortensen, Gillett-Kaufman JL, Ellis JD. 2015. Asian honey bee - *Apis cerana*. Entomology and Nematology Department, University of Florida, US.

Fine JD, Shpigler HY, Ray AM, Beach NJ, Sankey AL, Cash-Ahmed A, Huang ZY, Astrauskaite I, Chao R, Zhao H, Robinson GE. 2018. Quantifying the effects of pollen nutrition on honey bee queen egg laying with a new laboratory system. PLoS One 13 (9): e0203444. DOI: 10.1371/journal.pone.0203444.

Frunze O, Brandorf A, Kang E-J, Choi Y-S. 2021. Beekeeping genetic resources and retrieval of honey bee *Apis mellifera* L. stock in the Russian Federation: A review. Insects 12 (8): 684. DOI: 10.3390/insects12080684.

Hadisoesilo S, Kahono S. 2011. The potential of forest honey bee *Apis dorsata* in Sumbawa Besar. Expedition Report of Indonesian Forest Honey Association (JMHI). [Indonesian]

Haldhar SM, Nidhi CN, Singh KI, Devi AS. 2021a. Honeybees diversity, pollination, entrepreneurship and beekeeping scenario in NEH region of India. J Agric Ecol 12: 27-43. DOI: DOI: 10.53911/JAE.2021.12202.

Haldhar SM, Singh KI, Gupta MK, Devi AS. 2021b. Morphometric analysis on different species of honeybees in NEH region of India. In: Singh A, Sunder Barman J, Anand S. Jena P et al. (eds). International Web Conference on Global Research Initiatives for Sustainable Agriculture & Allied Sciences. Ashtha Foundation, Meerut, 13-15 December 2021.

Hassona N. 2017. Cubital index determination of some honey bee races *Apis mellifera* subspecies at El-Manzla region in El-Dakahlia Governorate, Egypt. J Plant Prot Pathol 8 (12): 625-629. DOI: 10.21608/jppp.2017.46930.

Hatjina F, Bieńkowska M, Charistos L, Chlebo R, Costa C, Dražić MM, Filipi J, Gregorc A, Ivanova EN, Kezić N, Kopernicky J, Kryger P, Lodesani M, Lokar V, Mladenovic M, Panasiuk B, Petrov PP, Rašic S, Skerl MIS, Vejsnæs F, Wilde J. 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. J Apic Res 53 (3): 337-363. DOI: 10.3896/IBRA.1.53.3.02.

He XJ, Zhou LB, Pan QZ, Barron AB, Yan WY, Zeng ZJ. 2017. Making a queen: An epigenetic analysis of the robustness of the honeybee (*Apis mellifera*) queen developmental pathway. Mol Ecol 26 (6): 1598-1607. DOI: 10.1111/mec.13990.

Ibrahim MM, Chandel YS, Anil. 2019. Morphometrics of *Apis cerana* from agroclimatic zones of Himachal Pradesh. Indian J Entomol 81 (3): 406. DOI: 10.5958/0974-8172.2019.00116.0.

Jagdale YD, Mahale SV, Zohra B, Nayik GA, Dar AH, Khan KA, Abdi G, Karabagias IK. 2021. Nutritional profile and potential health benefits of super foods: a review. Sustainability 13 (16): 9240; DOI: 10.3390/su13169240.

Ji C, Shi W, Tang J, Ji T, Gao J, Liu F, Shan J, Chen X, Chen C. 2023. Morphometrical analyses revealed high diversity of the eastern honey bee (*Apis cerana*) in mountains and islands in China. J Apic Res 62 (4): 647-655. DOI: 10.1080/00218839.2023.2205670.

Kahono S, Chantawannakul P, Engel MS. 2018. Social bees and the current status of beekeeping in Indonesia. In: Chantawannakul P, Williams G, Neumann P (eds). Springer Verlag, Berlin. DOI: 10.1007/978-981-10-8222-1_13.

Kaleka AS, Kaur N, Bali GK. 2019. Larval Development and Molting. In: Mikkola H (ed) Edible Insects. IntechOpen, London. DOI: 10.5772/intechopen.85530.

Kamyab S, Gharachorloo M, Honarvar M, Ghavami M. 2020. Quantitative analysis of bioactive compounds present in Iranian royal jelly. J Apic Res 59 (1): 42-52. DOI: 10.1080/00218839.2019.1673964.

Koetz AH. 2013. Ecology, behaviour and control of *Apis cerana* with a focus on relevance to the Australian Incursion. Insects 4 (4): 558-592. DOI: 10.3390/insects4040558.

Kuntadi K. 2013. Effect of larval ages on the quality of *Apis cerana* L. (Hymenoptera: Apidae) queens breed in captivity using queen-grafting technique. Jurnal Entomologi Indonesia 10 (1): 1-6. DOI: 10.5994/jei.101.1. [Indonesian]

Li Z, Hou M, Qiu Y, Zhao B, Nie H, Su S. 2020. Changes in antioxidant enzymes activity and metabolomic profiles in the guts of honey bee (*Apis mellifera*) larvae infected with *Ascosphaera apis*. Insects 11 (7): 419. DOI: 10.3390/insects11070419.

Mahbobi A, Farshineh-Adl M, Woyke J, Abbasi S. 2012. Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees (*Apis mellifera meda* Skorikov, 1929). J Apic Sci 56 (1): 93-98. DOI: 10.2478/v10289-012-0010-1.

Mattiello S, Rizzi R, Cattaneo M, Martino PA, Mortarino M. 2022. Effect of queen cell size on morphometric characteristics of queen honey bees (*Apis mellifera ligustica*). Ital J Anim Sci 21 (1): 532-538. DOI: 10.1080/1828051X.2022.2043790.

Metorima FN, Costa-Maia FM, Halak AL, Parpinelli RS, de Toledo VdeAA. 2015. Morphometric measurements of Africanized honeybee queens kept in an incubator or in queen banking. Acta Sci - Anim Sci 37 (1): 91. DOI: 10.4025/actascianimsci.v37i1.24006.

Minarti S, Yusryansyah AR, Masyithoh D. 2022. The effect of queen cell size difference in morphometrics of bees queen *Apis cerana*. J Ilmu-Ilmu Peternakan 32 (3): 373-379. DOI: 10.21776/ub.jiip.2022.032.03.08. [Indonesian]

Mubarok MFZ, Wadjdi MF, Puspitarini OR. 2020. Pengaruh berbagai ukuran sel ratu buatan terhadap larva lolos hidup, larva jadi pupa, dan panjang pupa pada lebah *Apis mellifera*. Jurnal Rekasatwa Peternakan 3 (1): 50-54. DOI: 10.33474/rekasatwa.v2i1.6359. [Indonesian]

Novita N, Saepudin R, Sutriyono S. 2013. Analisis morfometrik lebah madu pekerja *Apis cerana* budidaya pada dua ketinggian tempat yang berbeda. Jurnal Sain Peternakan Indonesia 8 (1): 41-56. DOI: 10.31186/jspi.id.8.1.41-56. [Indonesian]

Ostwald MM, Lyman BR, Shaffer Z, Fewell JH. 2020. Temporal and spatial dynamics of carpenter bee sociality revealed by CT imaging. Insect Soc 67 (2): 203-212. DOI: 10.1007/s00040-020-00761-w.

Pang C, Dong K, Guo Y, Ding G, Lu Y, Guo Z, Wu J, Huang J. 2022. Effects of three types of pollen on the growth and development of honey bee larvae (Hymenoptera, Apidae). Front Ecol Evol 10: 870081. DOI: 10.3389/fevo.2022.870081.

Rangel J, Böröczky K, Schal C, Tarpy DR. 2016. Honey bee (*Apis mellifera*) queen reproductive potential affects queen mandibular gland pheromone composition and worker retinue response. PloS One 11 (6): e0156027. DOI: 10.1371/journal.pone.0156027.

Resti Y, Dewi RK, Rayani TF. 2022. Temperature, humidity and light intensity in planting green fooder using a smart hydroponic system. Jurnal Sains Terapan 12 (2): 77-85. DOI: 10.29244/jstsv.12.2.77-85. [Indonesian]

Rocha I, Hoffmann A, Souto PM. 2022. Insect Morphology. In: Vonk J, Shackelford T (eds). Encyclopedia of Animal Cognition and Behavior. Springer, Cham. DOI: 10.1007/978-3-319-47829-6_1143-1.

Ropars L, Affre L, Geslin B. 2021. Morphometric identification of honey bee subspecies reveals a high proportion of hybrids within a Mediterranean protected area. J Apic Res 60 (5): 871-874. DOI: 10.1080/00218839.2021.1950973.

Sharma N, Vashisth, Sumit, Sharma, Pawan. 2013. Diversity and distribution of pests and predators of honeybees in Himachal Pradesh, India. Indian J Agric Res 48 (5): 392-401.

Shi YY, Huang ZY, Zeng ZJ, Wang ZL, Wu XB, Yan WY. 2011. Diet and cell size both affect queen-worker differentiation through DNA methylation in honey bees (*Apis mellifera*, Apidae). PLoS One 6 (4): e18808. DOI: 10.1371/journal.pone.0018808.

Suhri AGMI, Soesilohadi RCH, Putra RE, Raffiudin R, Purnobasuki H, Agus A, Kahono S. 2022. The effectiveness of stingless bees on pollination of bitter melon plants *Momordica charantia* L. (Cucurbitaceae). J Trop Biodivers Biotechnol 7 (3): 69124. DOI: 10.22146/jtbb.69124.

Ustadi, Ikhsan N, Junus M, Suyadi. 2022a. Improving the larvae acceptance rate and morphometric characteristics of queen *Apis cerana javana* by grafting larvae at different ages. Biodiversitas 23 (1): 166-170. DOI: 10.13057/biodiv/d230121.

Ustadi, Suyadi, Ikhsan N, Radiati LE, Sjofjan O, Susilo A, Batoro D. 2022b. The difference queen cup materials on the acceptance grafted larvae and wing morphometrics in *Apis cerana* queen rearing. In: Muhlisin, Umami N, Noviandi CT, Jayanegara A, Elsabagh M, Angeles AA, Keahavong B, Ortega ME (eds). Proceedings of the 6th International Seminar of Animal Nutrition and Feed Science (ISANFS 2021). DOI: 10.2991/absr.k.220401.051.

Wu X, Zhou L, Zou C, Zeng Z. 2018. Effects of queen cell size and caging days of mother queen on rearing young honey bee queens *Apis mellifera* L. J Apic Sci 62 (2): 215-222. DOI: 10.2478/JAS-2018-0025.