Effect of different queen cell sizes on the acceptance of grafted larvae in *Apis cerana javana* Fabr. Rearing

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**Abstract.** This study aims to evaluate *Apis cerana javana* Fabr. rearing based on differences in the queen cup sizes. A total of four queen bees were used with three different treatments and six replications, meanwhile, this study was conducted at Kembang Joyo Bee Farm, Malang, East Java. The results showed that the differences in the queen cup size had no significant effect on the success rate of queen rearing (p>0.05). The highest percentage of larvae acceptance (79%) was found in P3 treatment, followed by P2 (71%), while the lowest was found in P1 (63%). Furthermore, the highest percentage of larvae to pupae metamorphosis was found in P1 and P2 with a success rate of 100%, while the lowest was found in P3 with 89%. The highest percentage of pupae emergence was found in treatment P1 and P2 with a success rate of 100% and P3 with 94%. Based on the results, the difference queen cup sizes had no significant effect on the success rate of *Apis cerana javana* Fabr. rearing.

**Keywords:** Apis cerana, queen bee, queen cell size, queen rearing

1. **Introduction**

Bees are widely known to produce honey derived from plant nectar, meanwhile, the species mostly cultivated are *Apis cerana* and *Apis mellifera* [1]. Furthermore, honey bees are one of the most popular commodities in Indonesia [2]. The *Apis cerana* species are more cultivated due to the ability to adapt suitably to tropical climates, resist mites and parasites, moderately aggressive and are easy to breed [3]. This species is endemic to Asia and has been used for honey production and pollination for millennia [4].

*Apis cerana* bees are social insects that live in colonies. A honey bee colony typically consists of single queen bee (queen), hundreds of drones (drones), and thousands of female bees (workers). The queen bee is the most important member in her colony. The queen bee will lay eggs which in essence will produce worker bees and male bees. The quality of the queen bee also determines the production results that will be produced.
The high amount of production depends on the quality of the queen bee. If the queen bee is no longer productive or has low performance, requeening queen must be required to ensure maximum productivity [5]. A new queen bee can be produced by bee keepers using the queen rearing method. Moreover, several factors that influence in queen rearing including the size of queen cells, the material of queen cells, grafting techniques, age of larvae grafting and single grafting or double grafting method [6].

*Queen cell cups* is used for raise new queens and the range of natural size of *Apis mellifera* is about 8-10 mm [7]. The diameter at the rim of queen cell size should 8-9 mm and generally plastic queen cell size is 9.4 mm for *Apis mellifera* [8]. However, diameter queen cell at base is about 6.2 mm for *Apis cerana F.* which is more preferable by workers [6]. The difference size of queen cell cups affects success rate larvae into pupae and pupae to emerge *Apis cerana* [9]. In addition, queen cell size of *Apis cerana javana Fabr* has not been studied. Moreover, appropriate queen cells size will be easily accepted by workers.

Based on the description above, this study was conducted to determine the evaluation of rearing queen bee *Apis cerana javana* Fabr. based on the difference size of queen cells cup on the acceptance grafted larvae, larvae into pupae, pupae to emerge and the success rate grafting to emerge.

2. **Methods**

This is an experimental study carried out at the Kembang Joyo Honey Bee Farm, Donowarih Village, Karangploso District, Malang, East Java.

2.1. **Experimental colonies**

Thirteen honey bee colonies were prepared for this study, each containing 6 nest combs with sufficient honey and pollen deposits. The samples were divided into three different groups as follows:

*group (I)*

Six honey bee colonies (queenless) to collected natural queen cells and determine the suitable size of the artificial queen cells.

*group (II)*

Six queenless colonies for the experimental unit.

*group (III)*

One honey bee colony headed by queens to provide larvae supply.

2.2. **Producing artificial queen cells cup and collecting royal jelly**

Six different honey bee colonies without queen (queenless) were used to collect natural queen cells. Each colony contained six hive combs, young worker bees, young larvae, and sufficient honey and pollen. A total of 60 natural queen cells were carefully cut with a sharp cutter from the base, then the top and bottom diameter, as well as height of the cups at rim were measured and recorded. The larvae were removed to collect the royal jelly and stored in a freezer for the grafting process.

The natural queen cell sizes were classified into three groups namely small, medium, and large with nearly equal frequencies. Meanwhile, the average for each category was taken as the basis for production of artificial queen cell cups. The average height, as well as top and bottom diameters of small, medium and large natural queen cell cups size groups are presented in Table 1.

| No | Classifications | Height  | Top diameters | Bottom diameters |
|----|-----------------|---------|---------------|-----------------|
| 1  | Small           | 0.75 cm | 0.65 cm       | 0.44 cm         |
| 2  | Medium          | 0.85 cm | 0.71 cm       | 0.54 cm         |
| 3  | Large           | 1.00 cm | 0.71 cm       | 0.62 cm         |

Wooden dipping sticks with three different sizes that match each queen cell cup diameter category were carefully prepared and were used to produce artificial queen cell cup. Sufficient numbers of queen cell cups of each category were produced using pure *Apis cerana javana* Fabr. bees wax. The wax was
melted in a double jacket water bath at 65°C and the wooden tip of the mold was dipped in cold water for initial wetting. The wet end of the wood stick was dipped in wax and then removed (about 3-4 seconds). This process was repeated 5 times to get the desired thickness. The edges of the artificial queen cells cups that exceed the limit were trimmed with a cutter to make it uniform.

2.3. Grafting
A total of 72 one-day-old larvae were used to raise queen bees with 12 grafted larvae for each frame. Before grafting, a small drop (approx. 5 µL) of royal jelly was placed at the bottom of each of artificial queen cup [10].

2.4. Protection of queen cells and measurement
After extracting the queen cell from the colony for grafting, it was evident that worker bees added some new wax on the edges of the queen cell. In addition, observations for accepted larvae after 24 hours showed live larvae with royal jelly. The acceptance of grafted larvae was calculated by dividing the total accepted larvae by the total grafted, while the larvae to pupae percentage was calculated by dividing the total successful larvae to pupae by the total grafted. Observations made on the 6th day after larval grafting showed that the artificial queen cells were covered with wax indicating that the larvae entered the pupal stage. However, protection against attacks by worker bees is needed when the queen cells are left uncovered to prevent the escape of the queens, this is achieved by cell protectors or emergence cages. On the 12th day after grafting, the queen bees emerged from the respective cage, while pupae emergence was calculated by dividing the total emerged pupae by the total grafted larva. Furthermore, successful grafting was calculated by dividing the total emergence of grafted larvae by the total grafted.

2.5. Statistical analysis
This study was carried out using the Completely Randomized Design (CRD) method with 3 treatments and 6 replications. The data obtained were analyzed using the analysis of variance (ANOVA) with a significance level of 5%, while the treatments consist of three artificial queen cell cups of different sizes. Each treatment was tested in the experimental colony with a total of 12 artificial queen cell cups on the frame. Furthermore, a total of 6 *Apis cerana javana* Fabr. colonies were used as replications and the variables observed include acceptance of grafted larvae, larvae to pupae metamorphosis, emerged pupae, and successful rate of grafting.

3. Results and discussion

3.1. Acceptance of grafted larvae
The effect of differences in queen cell cup sizes on the acceptance of *Apis cerana javana* Fabr. larvae is presented in Table 2.

| Treatment | Success (%) |
|-----------|-------------|
| P1        | 63          |
| P2        | 71          |
| P3        | 79          |

The differences in queen cup sizes did not affect *Apis cerana javana* Fabr. larvae percentage of acceptance (P> 0.05) which ranged from 63% to 79%. The average yield was calculated from the number of successful grafting in each treatment [5]. The lowest percentage was found in treatment P1 with a small queen cup size (63%), while the highest (79%) was found in P3 with a large queen cup size. A previous study reported that the comfort level of worker bees in feeding royal jelly greatly affects the number of queen cells in the larvae [11]. The highest percentage was found in the medium-sized queen.
cups; this is in contrast to [11] which found the highest percentage in large-sized queen cups. This occurred due to differences in the characteristics of worker bees in each colony.

3.2 Larvae into pupae
The percentage of larvae into pupae against differences in the queen cup sizes is presented in Table 3.

| Treatment | Success (%) |
|-----------|-------------|
| P1        | 100         |
| P2        | 100         |
| P3        | 89          |

The difference in queen cup sizes did not affect the percentage of larvae into pupae (P> 0.05) which ranged from 89% to 100%. The lowest percentage (89%) was found in the P3 treatment with a large queen cup size, while the highest (100%) was found in P1 and P2 with small and medium queen cup sizes. A previous study reported that the success rate of larvae into pupae in *Apis cerana* reached 61% [5]. The metamorphosis of larvae into pupae is not significantly influenced by worker bees because pupae formations do not require food, therefore, the percentage of larvae into pupae is very high. Factors that influence the metamorphosis process during the observation include falling, inverting, and scratching of the larvae [11]. In this study, the different queen cup sizes provided a high percentage value for the larvae to pupae metamorphosis.

3.3 Pupae to emerge
The percentage of pupae to emerge against the differences in queen cup sizes is presented in Table 4.

| Treatment | Success (%) |
|-----------|-------------|
| P1        | 100         |
| P2        | 100         |
| P3        | 94          |

The difference in queen cup sizes did not affect the percentage of pupae emergence (P> 0.05) which ranged from 94% to 100%. The lowest percentage (94%) was found in the P3 treatment with a large queen cup size, while the highest (100%) was found in P1 and P2 with small and medium queen cup sizes, which both have a percentage of 100%. This is because the small size of the queen cells makes feeding easier for worker bees, hence, the success rate is increased [10]. The success rate of pupae emergence is strongly influenced by the metamorphosis of larvae into pupae. When the bee metamorphoses into pupae and is not eaten by predators, it emerges and becomes the queen. The low percentage in the large-sized queen cups is influenced by several factors, therefore, it is not suitable for use [12].

3.4 Grafting to emerge
The percentage of grafting to emerge due to the differences in queen cup sizes is presented in Table 5.

| Treatment | Success (%) |
|-----------|-------------|
| P1        | 63          |
| P2        | 71          |
| P3        | 67          |

The difference in queen cup size did not affect the percentage of successful grafting which ranged from 63% to 71% (P> 0.05). The success of the rearing process is measured from the grafting of larvae becoming queen and the productivity of the queen bees produced [5]. The lowest percentage (63%) was
found in treatment P1 with a small queen cup size, while the highest (71%) was found in P2 with a medium queen cup size. Several factors that influence the formation of queen bees include the size of the queen cup, the materials used in the manufacture of queen cups, the technique of grafting, the age of the larvae used, the media used for single or double grafting [6]. A previous study reported that medium queen bee cell sizes produced the best results compared to larger and smaller sizes [6]. Similarly, this study showed differences in the percentage of successful grafting.

4. Conclusion
Based on the results, differences in the sizes of the queen cup had no significant effect on the success rate of queen bee rearing, particularly on the acceptance of grafted larvae, larvae to pupae metamorphosis, pupae emergence, and the percentage of successful grafting.

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