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The Difference Queen Cup Materials on the Acceptance Grafted Larvae and Wing Morphometrics in *Apis cerana* Queen Rearing

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ABSTRACT
This study aims to determine the success rate and wings morphometric on *Apis cerana* queen rearing based on differences in wax sources (*Apis cerana*, *Apis mellifera, Apis dorsata* and their mixtures) as artificial queen cell cups. This study was used six replications and six treatments (P1: 100% *Apis cerana* wax; P2: 100% *Apis mellifera* wax; P3: 100% *Apis dorsata* wax; P4: 50% *Apis mellifera* wax + 50% *Apis cerana* wax; P4: 50% *Apis cerana* wax+50% *Apis dorsata* wax; P6: *Apis mellifera* wax+50% *Apis dorsata* wax). The results showed that the different sources of wax as a material of queen cell cups did not affect on the success rate of rearing *Apis cerana* queen bee (P>0.05). The highest percentage of larvae acceptance was found in the P5 treatment, namely 70% and the lowest found in the P1 and P3 treatments, namely 40%. The highest percentage of larvae to pupae was found in treatment P1, namely 75% and the lowest percentage of larvae to pupae was found in treatment P6, namely 20%. Moreover, the different sources of wax as a materials of queen cell cups did not affect on morphometric hind wing length, hind wing width, fore wing length, fore wing width, cubital (A, B), distance C, distance D, cubital index, radial field, inner wing length, inner wing width, dumbbell index, and wing radial cell (I, II, III, IV) queen bee *Apis cerana*. It can be concluded that the use of different beeswax as materials queen cups did not bring any effect on the success rate of rearing queen and wing morphometrics *Apis cerana*.

Keywords: *Apis cerana, morphometric, queen rearing, wax*

1. INTRODUCTION

Indonesia is a country with a tropical climate that is rich in biodiversity, both flora and fauna. Including the diversity of various species of honeybees. Honey bees in Indonesia can live freely in forest areas and several species can be cultivated.

In general, the beekeeping honey bee species in Indonesia are Apis cerana and *Apis mellifera* [1], while the *Apis dorsata* honey bee species [2] still live naturally in the forest and cannot be domesticated. Honey bees are one of the commodities that are the greater demand in Indonesia [3]. Indonesian people choose to cultivate *Apis cerana* and *Apis mellifera* honey bee species. *Apis cerana* bees can adapt to the tropical climate, are low resistant to mites and parasites, and are slightly aggressive and easy for beekeeping [4]. Compared to *Apis mellifera* species that are slightly susceptible to mite and parasitic attacks, this species is not aggressive, so it is easy to cultivate [5].

Apis cerana is one of nine genus Apis species (Family *Apidae*, Subfamily *Apinae*, Tribe *Apini*). *Apis cerana* is a major endemic to Asia and has been used as a bee for honey production and pollination for thousands of years [6]. *Apis cerana* bees are social insects that live in colonies. The classification caste in a bee colony consists of one queen bee (queen), hundreds of males bees (drones), and thousands of females/worker bees (workers). Each classification caste of members in a colony has a specific task and is organized in the colony.

The amount of high production depends on the condition of the queen bee. If the queen bee is no longer

productive or has decreased production, a queen bee must be replaced [7]. Queen bees can be made by beekeepers using the queen rearing method. The rearing process will significantly determine the queen bee's success, where optimizing queen bee production is essential to produce a prospective queen with better quality. [8] The success of queen bee rearing can be influenced by the basic ingredients queen cup, paraffin queen cups, old beeswax and fresh beeswax, was 0% 77.8% and 55.6% acceptance, respectively.

Several factors that can affect queen rearing include the size of the queen bee cells, the material of queen cells, grafting techniques or larvae grafting, age larvae used, single grafting or double grafting [8]. Based on the description above, this research was conducted to determine the success rate of rearing queen bee *Apis cerana* based on differences in beeswax sources as base material queen cups. Beekeepers can use this to get quality queens and be able to replace unproductive queen bees.

2. MATERIALS AND METHOD

Starting in August 2020, the study was conducted at Kembang Joyo Honey Bee Farm, Donowarih village, Karangploso district, Malang, East Java. The tools used in this research include rearing equipment, wooden frame and queen cup, grafting tool, queen bank, double jacket pan, caliper, photoshop. The materials used are wax from *Apis cerana* honey, *Apis mellifera*, *Apis dorsata*, *Apis cerana* worker bee larvae, carbon dioxide (CO2), honey and water

This research has 2 stages, namely preliminary research and primary research. The preliminary research was carried out to make a queen cup with a predetermined size based on the natural queen cells *Apis cerana*. The size of the Queen cup is 0.54 cm size with a bottom diameter, a top diameter of 0.71 cm and a length of 0.85 cm. Produce a queen cell cup using wooden sticks according to the specified size and mold by dipping the sticks into various melted waxes (according to treatment). A total of 72 larvae with one day age were used to raise queen bees grafting and 12 larvae for each frame. Before larvae were grafted, a small drop (approx. 5 µL) of royal jelly was placed at the bottom of each of the artificial queen cups [9]. After removing the queen cell from colony for grafting, it was evident that worker bees added some new wax on the edges of the queen cell.

After 24 hours, checking for accepted larvae was made. The accepted larva was indicated live larva and fed with royal jelly by worker bees. The acceptance of grafted larvae was measured by calculating the total of accepted larva devided by the total of the grafted larva. Larva to pupae was measured by calculating the total of successful pupae to emerge devided by the total of the grafted larva. Observations were done on 6th day after larval grafting when the artificial queen cells were covered with wax by worker bees. It indicated that the larvae entered the pupal stage. However, if queen cells are left to emerge in the nurse colonies, they have to be protected against attacks by workers and to prevent the escape of queens. Cell protectors or emergence cages can achieve this. On the 12th day after grafting the queen bees emerged in their respective emergence cages. Measuring was carried out immediately after the newly emerged queen bee was stunned with carbon dioxide gas.

Measurement morphometry of wing *Apis cerana* queen bee was done by a combination of photo and Photoshop programs 2021 series. The wing of *Apis cerana* queen bee was placed on an objective micrometer glass scale of 0.01 mm, then photographed perpendicularly and stored on the computer as an image file. The file was opened with the Photoshop program. The morphometry was measured by using the measuring tool menu, the scale listed on the objective micrometre.

The variable observed was hind wing length, hind wing width, fore wing length, fore wing width, cubital A (2-4), cubital B (1-2), distance C (3-4), distance D (11-15), cubital index (cubital A/B), radial field (0-7), inner wing length (1-14), inner wing width, radial field (0-7), inner wing length (1-14), inner wing width (7-13), dumbbell index (1-4/5-6), I (7-8), II (6-8), III (5-6), IV (0-5).

The number code for each wing morphometric measurement was shown in Figure 1.

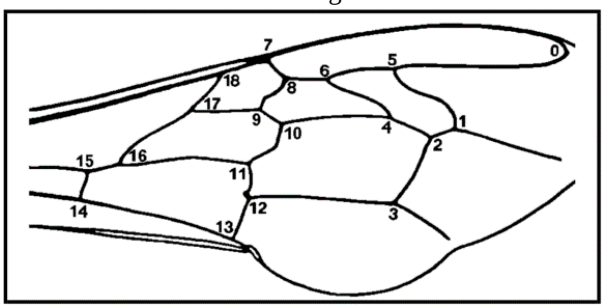

Figure 1. Morphometrics wing honey bee [10]

This experiment was used a Completely Randomized Design method (CRD) with 6 replications and 6 treatments. The treatments were used (P1: 100% *Apis cerana* wax; P2: 100% *Apis mellifera* wax; P3: 100% *Apis dorsata* wax; P4: 50% *Apis mellifera* wax + 50% *Apis cerana* wax; P4: 50% *Apis cerana* wax+50% *Apis dorsata* wax; P6: *Apis melliera* wax+50% *Apis dorsata* wax).

3. RESULTS AND DISCUSSIONS

3.1. Acceptance grafted larvae

Table 1. Acceptance grafted larvae dan percentage of larva into pupae *Apis cerana*

Treatments	Acceptance (%)	Larvae into pupae (%)
P1	40	75
P2	60	50
P3	40	50
P4	60	33
P5	70	57
P6	50	20

The results showed that the acceptance of *Apis cerana* larvae to differences in the queen cup can be seen in Table 1.

The use of different beeswax as materials queen cups did not affect on the acceptance of grafted larvae (P>0.05). The means of acceptance grafted larvae was 40%-70%. The average yield was calculated from the number of grafting success rates in each treatment of each experimental colony [7]. The lowest percentage of larvae acceptance was found in treatment P1 (100% *Apis cerana* beeswax) and P3 (100% *Apis dorsata* beeswax) namely 40%. The highest percentage of acceptance grafted larvae was found in treatment P5 (50% *Apis cerana* beeswax+ 50% *Apis dorsata beeswax)* namely 70%. The comfort level of worker bees in feeding royal jelly dramatically affects the number of queen cells in the successful acceptance of larvae [11]. This was possible due to differences in the characteristics of worker bees in each colony to find the level of comfort in feeding royal jelly.

3.2. Larvae Into Pupae

The results showed that the percentage of larvae into pupae to differences beeswax as a material of queen cell cups can be seen in Table 1. Different beeswax as materials queen cups did not affect the percentage of larvae becoming pupae (P>0.05). The means of percentage larvae into pupae was20%-75%. The lowest percentage larvae into pupae found in P6 treatments (50% *Apis mellifera* beeswax + 50% *Apis dorsata* beeswax) namely 20%. The highest percentage of larvae into pupae was found in P1 treatments (100% *Apis cerana* beeswax), namely 75%. Bees *Apis cerana*, the success rate of larvae into pupa reached 61% [7].

The process of changing larvae into pupa is not so influenced by feeding worker bees, because when bees become pupae, they do not need food, so the percentage of escaping larvae into pupa is very high. Factors that can influence the process of larvae becoming pupae during the observation process include falling pupae, inverted pupa, and scratching of the pupa causing failure to become a pupa [11]. This study showed that queen cups of various beeswax as base material queen cups were able to provide a high percentage value for the process of larvae becoming pupae.

3.3. Morfometrics Wing

The number code for each wing morphometric measurement can be seen in Figure 2.

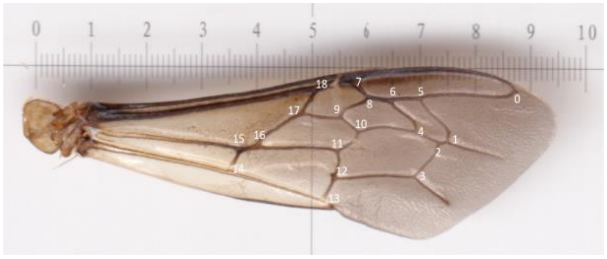

Figure 2. Queen bee wing parts

The morphometric measurements result of hind wing length, hind wing width, fore wing length, fore wing width, cubital A (2-4), cubital B (1-2), distance C (3-4), distance D (11-15), cubital index (cubital A/B), radial field (0-7), inner wing length (1-14), inner wing width (7-13), dumb-bell index (1-4/5-6), angle wing I, II, III, IV were presented in Table 2.

The result showed that using different beeswax as base material queen cups did not affect to the morphometric wings of *Apis cerana* queen bees (P<0.05) because the larvae were used from the same genotype, so it did not bring any differences in morphometric characteristics wing *Apis cerana* queen bee. Morphometric itself was used to identify characteristics morphology that described an overview of the diversity of honey bee genotypes. Table 2 shows the effect of differences in beeswaxes as base materials of queen cell cups on the wing morphometric.

4. CONCLUSIONS

Throughout the present study, it was found that the differences in beeswax as base materials of queen cups did not affect on acceptance of grafted larvae, larvae into pupae and morphometrics wing of queen bee *Apis cerana*. It can be concluded that wax from *Apis cerana*, *Apis mellifera*, *Apis dorsata* and their mixture can be used in rearing *Apis cerana* queen bee.

Table 2. Effect of differences beeswax as base materials of queen cell cups on the wing morphometrics

Morphological Characteristics (mm)	P1	P2	P3	P4	P5	P6
Hind Wing Length	6.3 ±0.44	6.1 ±0.01	6.0±0.17	6.0±0.28	6.2±0.39	6.2±0.12
Hind Wing Width	2.0±0.10	1.8±0.03	1.9±0.17	1.8±0.10	1.9±0.07	1.9±0.24
Fore Wing Length	8.3±0.09	8.1±0.34	8.2±0.29	8.1±0.54	8.3±0.22	8.4±0.13
Fore Wing Width	3.0±0.11	2.9±0.14	3.0±0.17	2.9±0.04	3.0±0.09	2.9±0.15
Cubital A (2-4)	0.4±0.02	0.4±0.03	0.4±0.02	0.4±0.05	0.4±0.03	0.4±0.03
Cubital B (1-2)	0.1±0.03	0.1±0.01	0.1±0.01	0.1±0.03	0.1±0.03	0.1±0.01
Distance C (3-4)	0.8±0.01	0.8±0.01	0.8±0.03	0.8±0.03	0.9±0.02	0.9±0.03
Distance D (11-15)	1.4±0.10	1.7±0.12	1.5±0.01	1.4±0.02	1.5±0.04	1.8±0.02
Cubital Index (A/B)	3.0±0.60	3.0±0.16	3.0±0.00	3.0±0.16	3.0±0.23	3.0±0.07
Radial Field (0-7)	2.8±0.07	2.8±0.07	2.8±0.07	2.8±0.05	2.8±0.39	2.7±0.20
Inner Wing Length (1-14)	4.0±0.03	3.9±0.07	4.1±0.04	3.9±0.08	4.1±0.06	4.0±0.03
Inner Wing Width (7-13)	2.1±0.02	2.1±0.00	2.1±0.02	2.1±0.00	2.1±0.04	2.1±0.00
Dumb Bell Index (1-4/5-6)	1.0±0.23	1.1±0.06	0.9±0.09	0.9±0.06	1.0±0.06	1.0±0.12
I (7-8)	0.3±0.15	0.3±0.07	0.3±0.07	0.3±0.00	0.3±0.09	0.3±0.09
II (6-8)	0.4±0.03	0.4±0.03	0.4±0.05	0.4±0.02	0.4±0.01	0.4±0.26
III (5-8)	0.5±0.04	0.5±0.02	0.5±0.00	0.5±0.03	0.5±0.02	0.5±0.014
IV (0-5)	1.7±0.08	1.5±0.08	1.6±0.01	1.5±0.02	1.6±0.02	1.7±0.01

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