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by Jasmi Jasmi

PROPAGATION OF *Apis cerana* FABR. (HYMENOPTERA: APIDAE) PROSPECTIVE QUEEN BEE

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ABSTRACT

Honey bee, *Apis cerana* Fabr. (Hymenoptera; Apidae) has been domesticated in West Sumatra. Various attempts to obtain practical and effective methods for multiplying superior colonies have been made. Optimizing the role of worker bees as a determinant of the future colony can be done by creating an emergency colony condition (queen bee not present). In this kind of colony, the worker bees immediately produced a new queen bee candidate from young larvae. This research was conducted at Trigona Mandiri Apiary, Padang Pariaman, West Sumatra, Indonesia from February to May 2019. The method used was a complete randomized design with four treatments and 10 replications. Results showed that the comb isolation technique was the best method for producing new queen bee candidates and increasing the appearance of young queen bee (gyn). The mean appearance of gyn from pupae was 4.8 per colony so that the number and quality of pupae to be maintained can be selected.

Key words: *Apis cerana*, worker, queen cell, emergency colony, colony multiplication.

ABSTRAK

Lebah madu, *Apis cerana* Fabr. (Hymenoptera; Apidae) telah dibiakkan di Sumatera Barat. Pelbagai usaha telah dilakukan untuk mendapatkan kaedah yang praktikal dan efektif dalam meningkatkan koloni-koloni unggul yang dipelihara. Mengoptimalkan penanan lebah pekerja sebagai penentu masa depan koloni dapat dilakukan dengan menciptakan koloni *emergency* (ratu lebah tidak hadir). Dalam koloni sebegini, lebah pekerja segera menghasilkan calon ratu lebah baru dari larva muda. Kajian ini telah dijalankan dari bulan Februari hingga Mei 2019 di Apiari Trigona Mandiri, Padang Pariaman, Sumatera Barat. Kaedah yang digunakan adalah melalui rekabentuk rawak dengan empat perlakuan dan 10 ulangan. Dari hasil kajian, diketahui bahawa teknik isolasi *comb* adalah kaedah terbaik untuk menghasilkan calon ratu lebah baru serta kemunculan ratu lebah muda (*gyn*). Rata-rata kemunculan *gyn* dari pupa adalah sebanyak 4.8 per koloni sehingga dapat dilakukan pemilihan terhadap jumlah dan kualiti pupa yang akan dipelihara.

INTRODUCTION

Honeybees are a group of social insects that form caste structures with specific functions in their colonies. The composition of the castes are queen bee, sterile female bees (worker bees) and male bees (Michener 1974; Wilson 1971). Each caste has distinct duties and functions so it cannot be replaced by a different caste. The division of duties in honey bee colonies is the most studied behavioral phenomenon. Research on the honey bees behavior has investigated since the 1800s, but serious experimental research only started in the early 1930s and continues until today with various laboratory approaches based on various biological perspectives (Beekman et al. 2007; Schmickl & Crailsheim 2007; Smith et al. 2008). This is because worker bees play an important role in determining the future of the colony such as preparing hives, food foraging even changing the sex of the larvae. The process of changing the sex of the larvae is carried out by worker bees if the colony is in an emergency condition that occurs if the colony lost the queen bee (Ribbands 1953).

Queen bee is the most important individual in a honeybee colony, because only queen bee that can produces both fertile and sterile eggs and produces pheromones to prevent worker bees from developing new queens and ovaries (Delaney et al. 2011; Winston 1987). Good quality queen bee is a fundamental factor for successful apiculture practices. A high-quality queen bee of *Apis mellifera* can be obtained by various methods such as isolation of queen bee (Tarpy et al. 2016), moving young larvae (12-24 hours old) from worker cells to queen bee cells that hung vertically in the same hive (Büchler et al. 2013), the Doolittle grafting method with wax and plastic bowls and utilization of the Cupkit and Karl Jenter apparatus (Dhaliwal et al. 2017). From those previous studies, the easy and practical methods were relatively few.

Apis cerana has an important role as a pollinator, especially for agricultural crops and orchards (Partap 2011; Sung et al. 2006) such as coffee plantation (Klein et al. 2003; Saepudin et al. 2011), *Jathropha curcas* (Atmowidi et al. 2008), *Mangifera indica* L. (Deuri et al. 2018) and *Prunus armeniaca* (Gurmani et al. 2016). In West Sumatra, this honeybee is easily found in lowland farming until altitude 1400m above sea level. Aside from being a pollinator, *A. cerana* also cultivated for producing honey. In West Sumatra, especially Padang Pariaman region, the cultivation of *A. cerana* was established from 1984 to the present. Honeybee colony are obtained by moving the wild colony to beehives by using the hiving method (Jasmi et al. 2014). The continuous poaching of wild *A. cerana* colony is directly affecting the presence and balance of insect's pollinator (Erniwati & Kahono 2009). Other factor that affect the wild *A. cerana* colony is the increase of wasp population, due to the wasp's predatory behavior (Arif et al. 2014; Omran et al. 2011).

Three common conditions in the natural maintenance of queen bee are loss of queen (resulted in an emergency colony), supersedure cells and swarming (Wade 2014). The principle of an emergency colony can be utilized to produce prospective queen bee. The manipulation of emergency conditions in superior colonies is very useful in producing new colonies that have the same relative qualities as the original colonies. Emergency conditions in bee colonies can be manipulated by various methods such as isolating the queen bee in *A. mellifera* (Tarpy et al. 2016), isolating combs (Büchler et al. 2013; Maramis & Rompas 2015; Tofilski & Czekonska 2004) and dividing the colony in *Tetragonula carbonaria* (Nunes et al. 2014). In addition to the emergency colony, queen bee production can also be stimulated by cutting the top of the combs (Puspitaningrum 1995; Snelgrove 1981). This study was focused on testing

the effectiveness of the isolation and comb cutting method in producing *A. cerana* queen bee cells.

MATERIALS AND METHODS

This study was conducted at Apiari Trigona Mandiri located in Kanagarian Batu Gadang Kuranji Hulu, Sungai Geringging District, Padang Pariaman, West Sumatra, Indonesia from February - May 2019. Geographically, the study site is located at 100' 07' 00" East longitude and 0' 33'00" South latitude (BPS 2018). The colonies were maintained by Apiary Trigona Mandiri originated from wild *Apis cerana* colonies that were transferred to the beehive. Colony age was varied from five to 30 months, but the colony used for the sample was ranged from five to six months. This study used 4 treatments and 10 replicates, thus a total of 40 colonies were involved. The treatments were comb isolation (A), comb-cutting with a triangle pattern (B), comb-cutting with a horizontal pattern (C) and control (D). The comb isolation method used refers to Büchler et al. (2013) whereas comb cutting following to Snelgrove (1981) and Stahlman (2013). Beehive size used in this study refers to Schouten et al. (2019) which is 40 x 30 x 20 cm. A beehive has nine frames with the area of each frame is 32 x 16 cm (Figure 2).

The colonies used in this study must be in a good condition, have exactly eight combs with a comb area in each frame more than 256 cm2 or >50% of the total frame space and at least there are three frames containing young brood cells (white-colored comb). If a colony has nine combs, one of the combs (preferably the broken or old one) was cut and the rest were put back into the beehive. All frames containing the combs were examined to ensure that there are no queen bee cells in all combs of the test colony. The results of beehive sorting were labeled on-site.

The treatment of comb insulation was done by placing the isolator between the 4th and 5th frames. The isolator was made from cardboard sized 35 x 26 x 0.3 cm (Figure 1). In each beehive there was nine frames (eight frames containing a comb and one empty frame). The isolator was divided and isolated the frame into two groups with four frames on the left (1st to 4th frame, along with the combs and adult bee occupying it) and five frame on the right (5th to 9th frame, along with the combs and adult bees occupying it and one empty frame) (Figure 2A & 2B). The model and size of the isolator was following the model and size of the beehive so that the isolator can play a role mainly to isolate the transfer of the queen bee from the left frame-group to the right frame-group or vice versa. After the isolator was installed, the beehive was closed.

Figure 1.   Model of comb isolation device used for propagation of *Apis cerana* queen bee
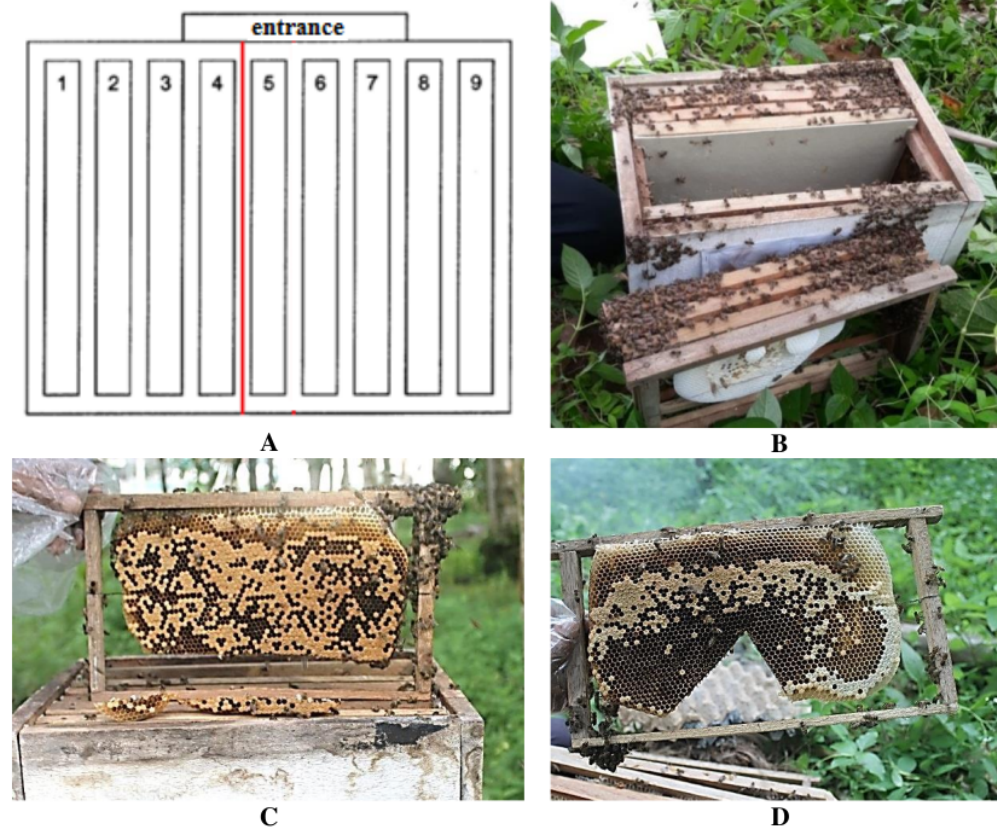
Figure 2.   The methods used to produces *Apis cerana* queen bee. (A) The isolator was located between the 4th and 5th frame, (B) the placement of the isolator between the 4th or 5th frame which already contains the comb, (C) comb with horizontal cutting pattern and (D) comb with triangle cutting pattern.

The frame chosen for the comb cutting method was the one with a minimum 75% comb area and have young combs which were marked with white or brownish wax. This process was carried out on 20 colonies by cutting the comb edge with two patterns. A total of 10 colonies were cut in a triangular pattern, 10 colonies horizontally and 10 other colonies were used as controls. The triangle pattern was made by cutting the comb from the top part to form the peak of the triangle in the middle of the comb at 50% height from the bottom of the frame (Figure 2D). The comb area cut following the triangle pattern was approximately 64 cm^2. Horizontal pattern was made by cutting the ¼ part from the comb edge (Figure 2C) or cutting the m-p area (Figure 3).
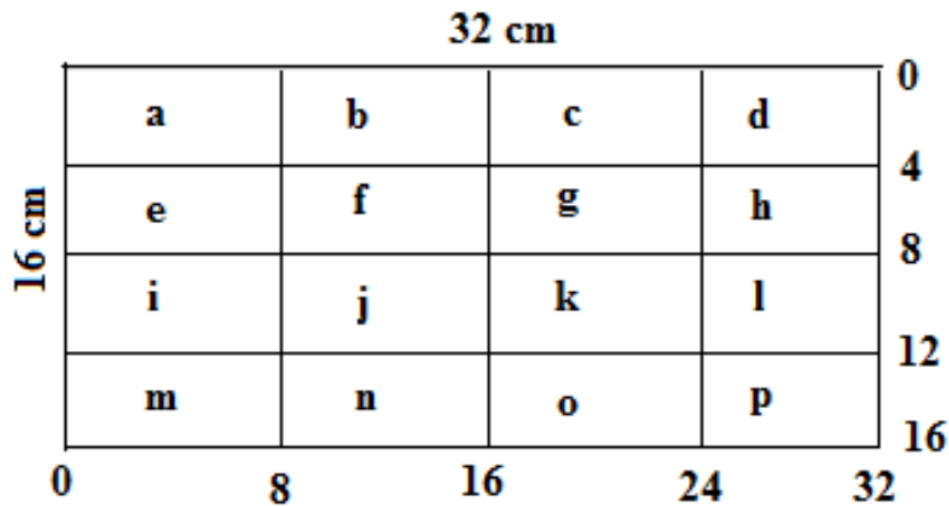
Figure 3.   The used frame model to estimate the increase in comb area of *Apis cerana*. a-p = sub-areas in a frame with an size of 32cm^2, equivalent to 6.25%

Examination of the prospective queen bee cells were carried out on each beehive comb for all treatment colonies on the 18th day. For the isolated colonies, the dismantling of isolator was done if the prospective queen cells were formed. All queen bee cells were allowed to develop naturally. An observation of the emerged pupae of prospective queen bee was done on the 24th day. The emergence of prospective queen bee pupae was marked by the abandoned pupae case.

Measured Parameters
Number of prospective queen bee cells
The number of prospective queen bee cells produced by each colony was calculated by checking the entire comb at the end of the observation (day 24th). The number of prospective queen bee cells counted was only normal queen bee cells (emergency cells and supersedure cells were not counted).

The total of emerged queen bee cells
Emerged queen bee cells were characterized by an open-top and empty cells. The number of emerged queen bee cells was determined for each comb per colony, following this formula,

Number of emerged queen bee cells
*   = number of prospective queen bee cells – number of emerged cells*

Area of the comb
The size of the used frame was 32 x 16 cm. To estimate the area of the comb formed in the frame, a frame model was made by dividing the frame area into 16 sub-area with size 32 cm^2 each (Figure 3). The model frame was attached to the frame containing the comb. The comb addition was calculated from the area of the newly formed comb on the frame from the cut comb and blank frames. Comb area (%) was estimated in the frame at the end of the observation

based on Delaplane et al. (2013) criteria by modifying the area of the frame into sub-areas (Figure 3).

Data Analysis

The treatment effects towards the total of prospective queen bee were analyzed using the one-way ANOVA Test (Analysis of Variance) followed by a post-hoc test using the Duncan test. The comb area size was expressed in % and analyzed using the chi-square formula. All analyses were performed using SPSS software version 21. Data for total emerged queen bee was expressed in percent (%) following the formula below,

$$\text{Percentage of the emerged prospective queen (\%)} = \frac{\text{Total emerged prospective queen bee}}{\text{Total of normal queen bee cells}} \times 100\%$$

RESULTS AND DISCUSSIONS

ANOVA results showed that there were differences between treatments on the comb size area and the average number of prospective queen bee cells formed from each sample colony ($P < 0.05$). Duncan's analysis results ($\alpha = 0.05$) showed that the largest comb average increase within a 18 days was found on the control colony, while the least was found on triangle pattern comb cutting treatment (Table 1). The highest average number of queen bee cells produced was found in the isolated treatment comb, while the least was found in comb cutting treatment.

Table 1.   The results of Duncan's analysis for an average increase in the area of *Apis cerana* (cm^2) combs and the number of prospective queen bee cells over a period of 18 days

Treatment	Total Samples	Measured Parameters	
		Comb area (cm^2)	Number of prospective queen bee (cell)
Comb isolation	10	38.40a	6.80b
Horizontal comb cutting	10	69.60b	0.00a
Triangle comb cutting	10	72.00 b	0.00a
No treatment (control)	10	133.60c	0.50a

* Different letters that indicate differences between treatments

Comb isolation is a method of dividing one bee colony into two groups, where one of the group has no queen bee, with the expectation to produce two independent colonies. The part of the colony without a queen bee is known as an emergency condition. The loss of the queen bee is a serious threat to the sustainability of the honeybee colony. In such condition, worker bees will play a reproductive-related role for the survival of the colony (Dixon et al. 2014). Worker bees plays an important role during the rearing phase of the larvae of prospective queen bee (AL-Kahtani 2011). According to Brouwers et al. (1987) during the stages of larval development, the worker bees will provide different food (in quality and quantity) for the cells of the prospective queen bee and worker bees (royal jelly vs worker jelly). This condition induced different development processes in these two cell types. As explained by Linksvayer et al. (2011), the control of nutritional factors is very important to larvae development, which is proven by the disappearance of dimorphism between the queen and the worker if the nutrients given to the larvae are the same.

The number of colonies produced the prospective queen bee cells were only 10 of 40 colonies, those were eight colonies from the comb isolation method and two colonies from control (Table 2). From 10 colonies that produced prospective queen bee cells, as many as seven colonies produced normal prospective queen bee cells, three colonies produced abnormal prospective queen bee cells and two colonies produced both types of cells. The number of frames occupied by prospective queen bee cells was only 26 out of the total 90 frames used. Out of these 26 frames, 17 frames are occupied by normal prospective queen bee cells, three frames are occupied by abnormal prospective queen bee cells and six frames have both types of cells.

Table 2.   Number of colonies that produce queen bee cells and combs are occupied by *Apis cerana* queen bee cells after 18 days of observation

Methods	Total sample colony	Total colony produced prospective queen bee cells		Total comb occupied by prospective queen bee cells	
		Normal	Abnormal	Normal	Abnormal
Triangle cutting pattern	10	0	0	0	0
Horizontal cutting pattern	10	0	0	0	0
Comb isolation	10	8	5	23	6
No treatment (control)	10	2	0	3	0
Total	**40**	**10**	**5**	**26**	**6**
Average	**10**	**2.5**	**1.75**	**7.00**	**1.50**

The propagation of the queen bee in *Apis cerana* was more effective with the comb isolation method (Table 2). The comb isolation method created an emergency condition for some part of the colony, then worker bees will immediately take action to produces prospective queen bee. Shi et al. (2011) stated that the emergency colony only had six days to produce a new queen after the last egg was produced by the old queen. If six day were passed, the egg has developed into a larva and loses its ability to develop into a functional queen (eggs hatch after 3 days and larvae under the age of 3 days still have totipotential ability).

As mentioned in the previous paragraph, the time required to produce *A. cerana* prospective queen bee in an emergency colony was relatively short. Emergency conditions will affect the behavior of worker bees in determining the future of the colony by producing new prospective queen bee. Büchler et al. (2013) reported that the success and quality of queen bee production depend on the strength and adequacy of the nutrition of a colony, as well as the quality of colony management. The important thing in raising queen bees for emergency colonies according to Woodward (2007) is the process of transferring 12-24 hour old larvae from worker cells to queen cells located vertically in the middle of the hive. These larvae will be fed royal jelly by worker bees. Royal jelly is a high-quality food source that can change the sex of the bee while it is still in the larval phase for less than three days. Rafique et al. (2019) reported that the addition of royal jelly in prospective queen bee cells before the new larvae grafted was more effective compared to the addition during the queen rearing process.

The distribution of normal queen bee cells were on frames 1–8 (Table 3). A total of eight colonies separated by an insulator (8 parts on the left and 8 parts on the right) were produced normal queen bee cells. All parts of the colony that were separated by an insulator can produce prospective queen bee cells both from the part of the colony (with or without queen

bee). The total number of normal prospective queen bee cells was higher in the frame located to the left side of the insulator (46 cells) compared to the right side (22 cells).

Table 3.   Distribution of normal queen bee cells found on each comb of 10 *Apis cerana* colonies on 18th observation day

Method	Colony code	Comb (frame) number per total prospective queen bee cells									Total
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
Comb isolation	1	0	3	3	4	3	1	0	1	0	15
	2	0	3	2	4	2	2	0	0	0	13
	3	0	0	1	2	1	0	0	0	0	4
	6	0	1	8	2	3	1	2	0	0	17
	7	0	1	3	0	1	0	0	0	0	5
	8	1	0	2	1	1	0	0	0	0	5
	9	0	2	1	2	0	1	0	0	0	6
	10	0	0	0	0	0	2	1	0	0	3
	Summary	**1**	**10**	**20**	**15**	**11**	**7**	**3**	**1**	**0**	**68**
	Average	**0.22**	**2.22**	**4.44**	**3.33**	**2.44**	**1.56**	**0.67**	**0.22**	**0.00**	**15.11**
Control	5	0	0	0	1	0	1	0	0	0	2
	8	0	1	0	0	2	0	0	0	0	3
	Summary	**0**	**1**	**0**	**1**	**2**	**1**	**0**	**0**	**0**	**5**
	Average	**0.00**	**0.50**	**0.00**	**0.50**	**1.00**	**0.50**	**0.00**	**0.00**	**0.00**	**2.50**
Total		**1**	**11**	**20**	**17**	**13**	**8**	**3**	**1**	**0**	**73**

The highest number of normal prospective queen bee cells was found in the 3rd frame (20 cells) and 4th frame (15 cells), while no cells found on the 9th frame. The highest number of normal queen bee cells in one frame was 10 cells which were consisted of eight normal cells and two abnormal cells (Figure 4b). The highest number of normal prospective queen bee cells was found in the comb isolation method which was 7 cells per frame. The highest number of normal queen bee cells produced by one colony was 17 cells per colony.

Prospective queen bee cells were placed in all the comb area categories (Table 4). The average number of prospective queen bee cells produced was 18.25±33.25 cells per treatment or 1.82±0.83 cells per colony. The largest number of queen bee cells was found in the comb area >384 cm^2 (comb area covers >75% of frame space) which was 41 cells and at least in the comb area less than 128 cm^2 (comb area covers <25% of frame space) which was 2 cell. The wider the comb in the isolation treatment, the more number of queen bee cells were found. For the comb cutting treatment no queen bee cells were produced within the 18 days observation period.

Table 4.   Number of prospective queen bee cells found in various groups of comb area from 10 *Apis cerana* colonies after 18 days of treatment

Method	Comb area (cm^2) per total number of prospective queen bee cells				Total cells of prospective queen bee
	>384	256-384	129-256	<128	
Triangle cutting pattern	0	0	0	0	0
Horizontal cutting pattern	0	0	0	0	0
Comb isolation	37	23	6	2	68
No treatment (control)	4	1	0	0	5
Total comb	**41**	**24**	**6**	**2**	**73**
Stdev for treatment	**17.93**	**11.34**	**3.00**	**1.00**	**33.25**
Average per colony	**1.02**	**0.6**	**0.15**	**0.05**	**1.82**
Stdev per colony	**0.45**	**0.28**	**0.08**	**0.03**	**0.83**

The comb structure occupied by the prospective queen bee cells was varied. Prospective queen bee cells were placed on a comb that occupied by brood cells, food storage and empty cells (Figure 4). Some queen bee cells were also placed on the same comb as the worker and drone/ male bee brood cells (Figure 4a and 4b), drone brood cells only (Figure 4d) and worker bee brood cells only (Figure 4c). Prospective queen bee cells were also found on the same comb as food storage cells containing pollen and honey (Figure 4a) and pollen only (Figure 4d). There are also queen bee cells that are placed on a comb with only empty cells (Figure 4c).
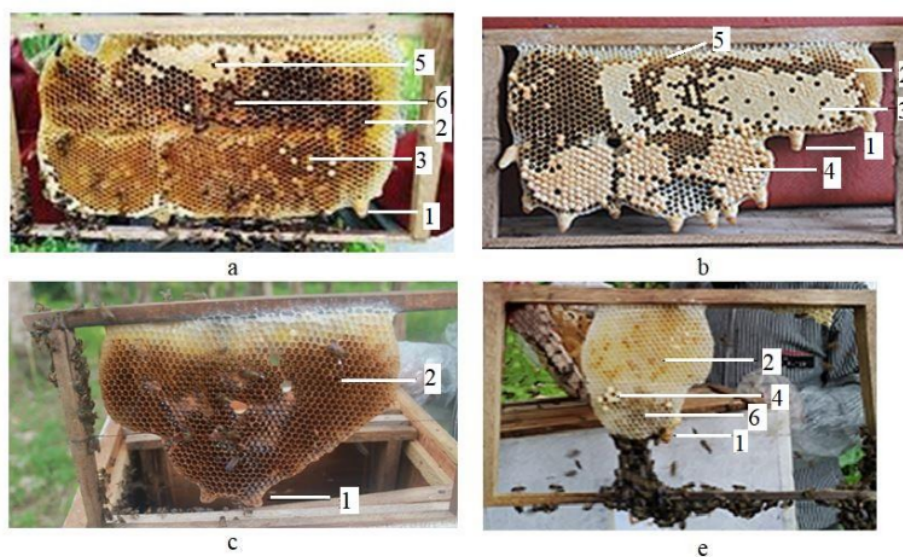

Figure 4.   Some area and cells conditions of *Apis cerana* combs occupied by prospective queen bee cells after 18 days of observation. (a) The comb area was >384 cm^2 occupied by prospective queen bee, worker bees and male bees brood cells, food

storage and empty cells. (b) The comb area was 256-384 cm^2 occupied by prospective queen bee, worker bees and male bees brood cells, food reserves and empty cells. (c) The comb area was 129-256 cm^2 occupied by prospective queen bees and worker bee brood cells also empty cells. (d) The comb area was <128 cm^2 occupied by prospective queen bees brood cells, pollen cells and empty cells.

1 = queen cell, 2 = empty cell, 3 = worker pupa cell, 4 = drone pupae cell, 5 = honey cell, 6 = pollen cell.

The Total Prospective Queen Bee Cells that Emerged as New Queen Bee (Gyn)
The total number of prospective queen bees emerged as young queen bee (gyn) on the 24th day was 71.23% which consisted of 65.75% from comb isolation treatment and 5.48% from control colonies (Table 5). The success of emerged pupae to gyn was higher (100%) in colonies with fewer queen bee cells. A higher percentage of non-emerged pupae (>30%) was found in colonies that had more than 5 prospective queen bee cells.

Table 5. Nmber of *Apis cerana* prospective queen bee cells that emerged on the 24th observation day

Method	Colony code / number	Total emerged pupae of prospective queen bee/ colony		
		Pupae	**Emerged**	**Percentage of (%) emergence**
Comb isolation	1	15	11	73.33
	2	13	9	69.23
	3	4	3	75.00
	6	17	11	64.70
	7	5	3	60.00
	8	5	4	80.00
	9	6	4	66.66
	10	3	3	100.00
	Summary	68	48	588.92
	Average	8,5	6	73.62
	Stdev	5.55	3.66	12.37
No treatment (control)	5	2	2	100.00
	8	3	2	66.66
	Summary	5	4	166.66
	Average	2.5	2	83.33
	Stdev	0.71	0.00	23.57
Total		**73**	**52**	**71.23**

The percentage of emerged pupae using the comb isolation method was 65.75% (Table 5). The success of the young bees' emergence will vary for each method and type of bee, for example in *Apis mellifera* with cupkit queen rearing technique the percentage of emergence was 54.67% (Dhaliwal et al. 2017) whereas Doolittle grafting method the queen bee emergence percentage was 53% and 77% (Emsen et al. 2003). The usage of in-vitro rearing method with

the addition of a highly concentrated sugar solution also able to increase the number of the prospective queen bee (Kaftanoglu et al. 2011).

The new queen bees were found at 18th observation day in a colony which had 17 cells of prospective queen bee cells (Figure 5). Prospective queen bee that emerged as gyn was marked by the opening of the lid (cup) at the top of the pupae cell. The wings of newly emerged queen bee appeared a bit tangled but slowly the wings will dry and look tidy. Prospective queen bee cells that were failed to emerge were contained of dead pupae in damaged condition.
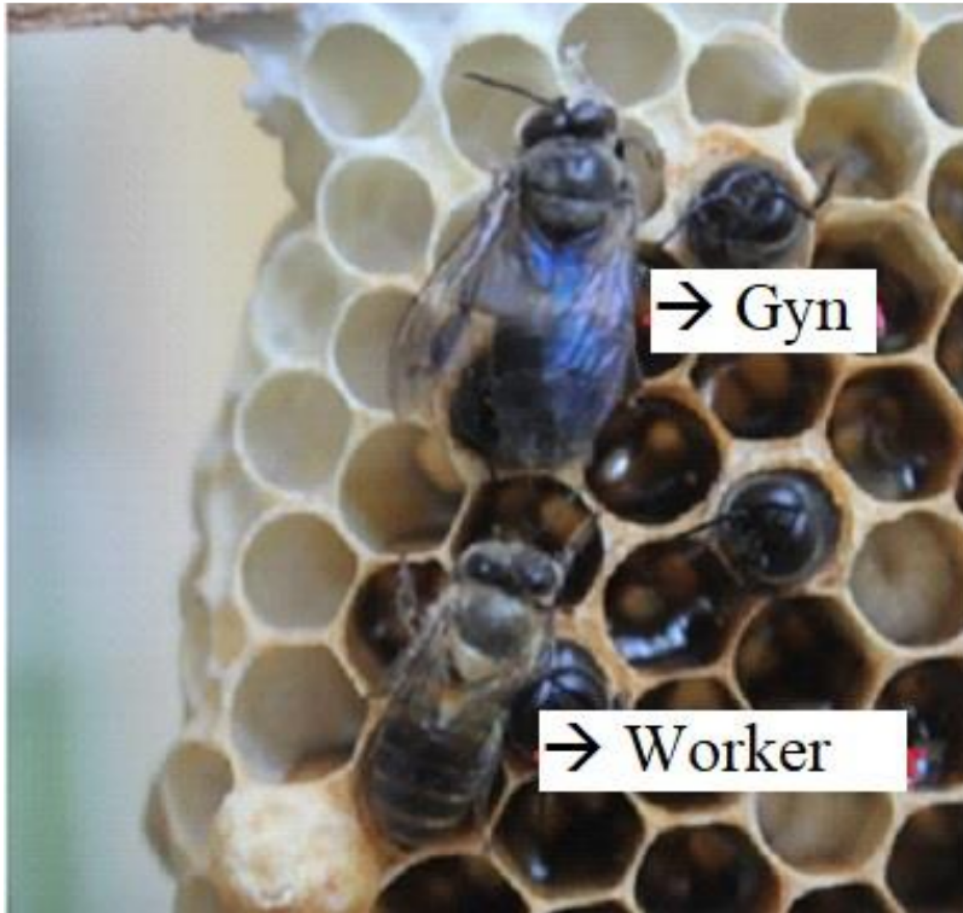

Figure 5.   Newly emerged young queen bee (*gyn*) of *Apis cerana* on the colony with an initial comb of nine frames

The comb isolation method is more effective to produce a high number of prospective queen bee cells (Table 5). One colony can produce as many as 17 prospective queen bee cells, where in one comb 8 normal prospective queen bee cells can be found (Figure 5). The emergence of relatively high number of prospective queen bee in a single colony can be caused by various factors such as colonies in emergency conditions (Tarpy et al. 2016; Tofilski & Czekonska 2004) and artificial prospective queen bee cells through various methods (AL-

Kahtani 2011; Dhaliwal et al. 2017; Emsen et al. 2003; Kuntadi 2013; Okuyan & Akyol 2018; Rafique et al. 2019).

CONCLUSION

In this study the comb isolation technique was the best method for producing new prospective queen bees and to increasing the appearance of young queen bees. Comb isolation is an excellent technique for the apiary with difficulties in practicing colony propagation. The average appearance of young queen bees (gyn) from pupae was 4.8 per colony so that the number and quality of pupae to be maintained can be selected.

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REFERENCES

AL-Kahtani, S. N. S. 2011. Selection of larvae for queen rearing by workers in the honey bee (*Apis mellifera* L.) − *Nepotism or different maternal supply*? *Dissertation*, Humboldt-Universität zu Berlin.

Arif, M. M. J., Norashirene, M. J., Khairiyah, M. H. S., Nicholas, A. S., Aman, A. Z., Abdul-Latiff, M. A. B., Aifat, N. R., Yaakop, S. & Yusof, M. D. F. Z. 2014. Initial study of social vespid wasps by using molecular approach of 28s region. *Serangga* 19(1): 51-61.

Atmowidi, T., Riyanti, P. & Sutrisna, A. 2008. Pollination effectiveness of *Apis cerana* Fabricus and *Apis mellifera* Linnaeus (Hymenoptera: Apidae) in *Jathropha curcas* L. (Euphorbiaceae). *Biotropia* 15(2): 129-134.

Beekman, M., Gilchrist, A. L., Duncan, M. & Sumpter, D. J. 2007. What makes a honeybee scout? *Behavioral Ecology and Sociobiology* 61(7): 985-995.

BPS [Badan Pusat Statistik]. 2018. *Statistics of West Sumatra in 2018*. Padang: The central statistics agency of west sumatra province [Indonesia].

Brouwers, E. V. M., Ebert, R. & Beetsma, J. 1987. Behavioural and physiological aspects of nurse bees in relation to the composition of larval food during caste differentiation in the honeybee. *Journal of Apicultural Research* 26(1): 11-23.

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. *Journal of Apicultural Research* 52(1): 1-30.

Delaney, D. A., Keller, J. J., Caren, J. R. & Tarpy, D. R. 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). *Apidologie* 42(1): 1-13.

Delaplane, K. S., Van Der Steen, J. & Guzman, E. 2013. Standard Methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research* 52(1):1-12.

Deuri, A., Rahman, A., Gogoi, J., Borah, P. & Bathari, M. 2018. Pollinator diversity and effect of *Apis cerana* F. pollination on yield of mango (*Mangifera indica*). *Journal of Entomology and Zoology Studies* 6(5): 957-961.

Dhaliwal, N. K., Singh, J. & Chhuneja, P. K. 2017. Comparative evaluation of Doolittle, Cupkit and Karl Jenter techniques for rearing *Apis mellifera* Linnaeus queen bees during breeding season. *Journal of Applied and Natural Science* 9(3): 1658 -1661.

Dixon, L. R., Kuster, R. D. & Rueppell, O. 2014. Reproduction, social behavior, and aging trajectories in honey bee workers. *AGE* 36(1)*:* 89-101.

Emsen, B., Dodologlu, A., Genc, F., Smith, B. H. & Cobey, S. 2003. Effect of larvae age and grafting method on the larvae accepted rate and height of sealed queen cell *(Apis rnellifera* L.). *Journal of Applied Animal Research* 24 (2): 201-206.

Erniwati & Kahono, S. 2009. The role of wild plants in the conservation of pollinating insect order Hymenoptera. *Jurnal Teknik Lingkungan* 10 (2): 195-203. [Indonesia]

Gurmani, H. T. W., Jaleel, W., Saeed, S., Gurmani, A. R., Corresp, Q. S., Naqqash, M. N., Ghouri, F., Munawar, M. S., Sial, M. U., Rahman, S. & Aine, Q. U. 2016. The pollination potential of *Apis cerana* Fab., *Apis mellifera* L. (Hymenoptera: Apidae) on yield of apricot (*Prunus armeniaca*). *Peer Journal Preprints* 1-19.

Jasmi, Salmah, S. & Dahelmi. 2014. Nesting site *Apis cerana* Fabr. (Hymenoptera: Apidae) in two different altitude of polycultureplantations in West Sumatra. *Hayati Journal of Bioscience* 21 (3): 135-143.

Kaftanoglu, O., Linksvayer, T. A. & Page, R. E. 2011. Rearing honey bees, *Apis mellifera*, *in vitro* 1: effects of sugar concentrations on survival and development. *Journal of Insect Science* 11(96): 1-12.

Klein, A-M., Steffan-Dewenter, I. & Tscharntke, T. 2003. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). *American Journal of Botany* 90 (1): 153-157.

Kuntadi. 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. [Indonesia].

Linksvayer, T. A., Kaftanoglu, O., Akyol, E., Blatch, S., Amdam, G. V. & Page Jr, R. E. 2011. Larval and nurse worker control of developmental plasticity and the evolution of honey bee queen-worker dimorphism. *Journal of Evolutionary Biology* 24(9): 1939-1948.

Maramis, R. T. D. & Rompas, J. J. I. 2015. Utilizing the queen rearing method of *Apis mellifera* for beekeping development. *Zootek* 35 (2): 235-246. [Indonesia].

Michener, C. D. 1974. *The Social Behavior of the Bees a Comparative Study*. Cambridge. Massachusetts: The Belknap Press of Havard University.

Nunes, T. M., Heard, T. A., Venturieri, G. C. & Oldroyd, B. P. 2014. Emergency queens in *Tetragonula carbonaria* (Smith, 1854) (Hymenoptera: Apidae: Meliponini). *Austral Entomology* 54(2): 154-158.

Okuyan, S. & Akyol, E. 2018. The effects of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. *Turkish Journal of Agriculture - Food Science and Technology* 6(11): 1556-1561.

Partap, U. 2011. The pollination role of honeybees. In Hepburn, H. R. & Radloff, S. E. (eds.) *Honeybees of Asia*, pp. 227-255. Heidelberg: Springer-Verlag Berlin.

Puspitaningrum, W. I. 1995. The effect of comb cutting on the number of queen cells built by *Apis cerana* honeybees. Undergraduate Thesis. FMIPA. Universitas Diponegoro. Semarang. [Indonesia]

Omran, N. S. M., Hussein, M. H., Khodairy, M. M. & Awad, A. M. 2011. Predators of honeybee and its impact on activities of honeybee colonies under conditions of South Valley, Egypt. *Journal Agricultural and Biological Science* 7(1): 79-88.

Rafique, M. K., Mahmood, R., Qadir, Z. A., Bodlah, I. & Shaheen, F. A. 2019. Effects of rearing interlude and grafting technique on honeybee *Apis mellifera* L. queen under field conditions. *Pakistan Journal of Zoology* 51(6): 1-4.

Ribbands, C. R. 1953. *The Behavior and Social Life of Honey Bees*. London: Bee Res. Assn.

Saepudin, R., Fuah, A. M. & Sumantri, C. 2011. Increased productivity of honeybee through the application integration with coffee plantations. *Jurnal Ilmu-Ilmu Peternakan* 21: 29-39. [Indonesia]

Schmickl, T. & Crailsheim, K. 2007. HoPoMo: A model of honeybee intracolonial population dynamics and resource management. *Ecological Modelling* 204(1-2): 219-245.

Schouten, C., Lloyd, D. & Lloyd, H. 2019. Beekeeping with the asian honey bee (*Apis cerana javana* Fabr) in the Indonesian islands of Java, Bali, Nusa Penida, and Sumbawa. *Bee World* 1-5.

Shi, Y. Y., Huang, Z. Y., Zeng, Z. J., Wang, Z. L., Wu, X. B. & Yan, W. Y. 2011. Diet and cell size both affect queen-worker differentiation through DNA methylation in honey bees (*Apis mellifera*, Apidae). *PLoS ONE* 6(4): e18808.

Smith, C. R., Toth, A. L., Suarez, A. V. & Robinson, G. E. 2008. Genetic and genomic analyses of the division of labour in insect societies. *Nature Review Genetics* 9:735-748.

Snelgrove, L. E. 1981. *Queen* Rearing. 4th Edition. Bleadon Hill, Weston-Super Mare, Avon: Snelgrove & Smith.

Stahlman, D. 2013. *A Queen Manual*. London: Department of Agriculture Division of Plant Industry- Apiary.

Sung, I. H., Lin, M. Y., Chang, C. H., Cheng, A. S. & Che, W. S. 2006. Pollinator and their behaviors on mango flowers in Southern Taiwan. *Formosan Entomol* 26: 161-170.

Tarpy, D. R., Simone-Finstrom, M. & Linksvayer, T. A. 2016. Honey bee colonies regulate queen reproductive traits by controlling which queens survive to adulthood. *Insectes Sociaux* 63(1): 169-174.

Tofilski, A. & Czekonska. K, 2004. Emergency queen rearing in honeybee colonies with brood of known age. *Apidologie* 35(3): 275-282.

Wade, A. 2014. *Queen Rearing and Use of Queens*. Cambera: Beekeepers Association of the ACT (Department of Primary Industries).

Wei, S., Raina, S. K. & Fries, I. 2001. Colony development and queen rearing in Kenyan honeybees (*Apis mellifera scutellata*). Durban, South Africa. Proc. 37th Int. Apic. Congr. 28 Oct-1 Nov 2001.

Wilson, E. O. 1971. *The Insect Societies*. Cambridge: Harvard University Press.

Winston, M. L. 1987. *The Biology of the Honey Bee*. Cambridge: Harvard University Press.

Woodward, D. 2007. *Queen Bee: Biology, Reading and Breeding*. New Zealand: Balclutha.

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