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Nest Characteristics of Stingless Bee *Heterotrigona itama* (Hymenoptera: Apidae) upon Colony Transfer and Splitting

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ABSTRACT

Stingless bees are in a diverse group of highly eusocial bees (meliponines) which are reared for their products and pollination potentials. The main source of stingless bee colonies is depending on feral colony hunting that potentially affects the ecosystem. Therefore, establishment of a good practise in colony transfer and splitting may extend the survival of a colony in a new location. This study describes a colony transfer strategy from log to hive and nest characteristics of stingless bee *Heterotrigoma itama* upon colony splitting from the hive. All 15 colonies from the hollow trunks of trees could survive after one month of transfer to hives. Pre-colony splitting data of 10 daughter colonies showed that the height of brood cells was 14.5 ± 1.20 cm, the number of brood layer was 8.9 ± 2.13, while the number of queen cell was 5.2 ± 1.32. After 12 weeks, the height of brood cells became 16.42 ± 1.05 cm, while the numbers of brood layer and virgin queen eggs were increased to 12.1 ± 1.85 and 6.4 ± 1.65, respectively. However, the correlation between the number of brood layer and virgin queen egg was significantly low (r = 0.421). These results show the ideal strategy for colony transfer from log and colony splitting, and the number of brood layer does not correlate to the presence of virgin queen egg which is crucial for survival of the colony.

Keywords: Colony splitting, *Heterotrigona itama*, stingless bee

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INTRODUCTION

Stingless bee (Meliponini) are eusocial insects which live in a colony consist of a single queen which controls thousands of workers. Similar to honeybee (*Apis*), stingless bee exhibits complex social, navigational and communication behaviours (Kärcher et al., 2013). In social insect colonies, the duration for the colony development process will influence the community expansion together with the substitution of the workers. These will affect the colony's ability to assemble and supply nutrient assets, which will then influence the colony's condition, dominance and duration to survive (Halcroft et al., 2013). The duration of life for an insect is mostly based on food uptake (Haydak, 1970), thus proper nursing in the early stage of life will increase the lifespan of juvenile stingless bee (Carey, 2001). Unlike Apini, the slower digestion of food by Meliponini larvae may affect its colony growth rate (Moo-Valle et al., 2004).

Meliponini can easily be found in tropical regions globally (Heard, 1999). In Malaysia, about 45 species of stingless bee were reported until 2014 (Jaapar et al., 2016). The *Heterotrigona itama* and *Genotrigona thoracica* are among the main species being reared individually or commercially (Kelly et al., 2014; Mustafa et al., 2018). As the valuable physicochemical, antioxidant and antimicrobial properties of the Malaysian stingless bee honey have gradually been recognized recently (Bakar et al., 2017; Ranneh et al., 2018; Tuksitha et al., 2018), the demand has also increased. The current market price of stingless bee honey in Malaysia reaches RM85 per 150 g.

The name stingless bee portrays the underdeveloped stinger (Shackleton et al., 2015), which makes this species less harmful than the stinger bees. Due to this factor, meliponiculture or stingless bee beekeeping activity has become a trend in many tropical countries (Cortopassi-Laurino et al., 2006). Traditionally, the tree trunks which contain the wild colonies are cut and relocated to a bee farm. Besides promoting deforestation and destruction of forest due to illegal hunting for stingless bee logs (Sommeijer, 1999), keeping the colonies in their own tree trunks restricts the observation of the internal structure and condition. To achieve a successful domestication, a good practice in transferring the colonies from logs into artificial house (hive) and performing colony splitting or multiplication may help to sustain the ecosystem (Singh, 2013). Colony splitting requires proper skills and wide knowledge on the insects' behaviour as it involves appropriate time, season and ability of the personnel to identify the perfect condition and location of the colony to be split (Cortopassi-Laurino et al., 2006). The new colony that is obtained from a splitting technique is relatively weak and vulnerable towards its natural threat such as ants, as the nursery process upon splitting mainly involves ensuring sufficient food to the new colony, rather than protection (Jaffé et al., 2015).

A few bee hive constructs has been patented and utilised successfully (Quezada-Euan et al., 2001). However, scientific documentation on colony transfer and splitting techniques and nest structure of economically-important stingless bee species of Malaysia, such as *H. itama,* is relatively scarce (Saufi & Thevan, 2015). Therefore, this study explores those limitations and analyses the nest characteristics of the colonies upon splitting.

MATERIALS AND METHODS

Selection of Hive and Colony

All procedures were carried out at a bee farm located at Tumpat, Kelantan, Malaysia (6^{o} 8’ 26.5’’N; 102^{o} 13’ 15.9’’ E). A total of 15 colonies of *H. itama* was used for colony transfer from logs to hives. The MUSTAFA-Hive used in this study was designed by Dr. Mohd Zulkifli Mustafa (Jalil & Roubik, 2017).

Colony Transfer Technique from Log to Hive

Data on nest entrance and height of tree trunk with wild colony by Kelly et al. (2014) were referred. The hollow tree trunks with wild colonies (*n*=15) were removed with care, accordingly (Kelly et al., 2014). The

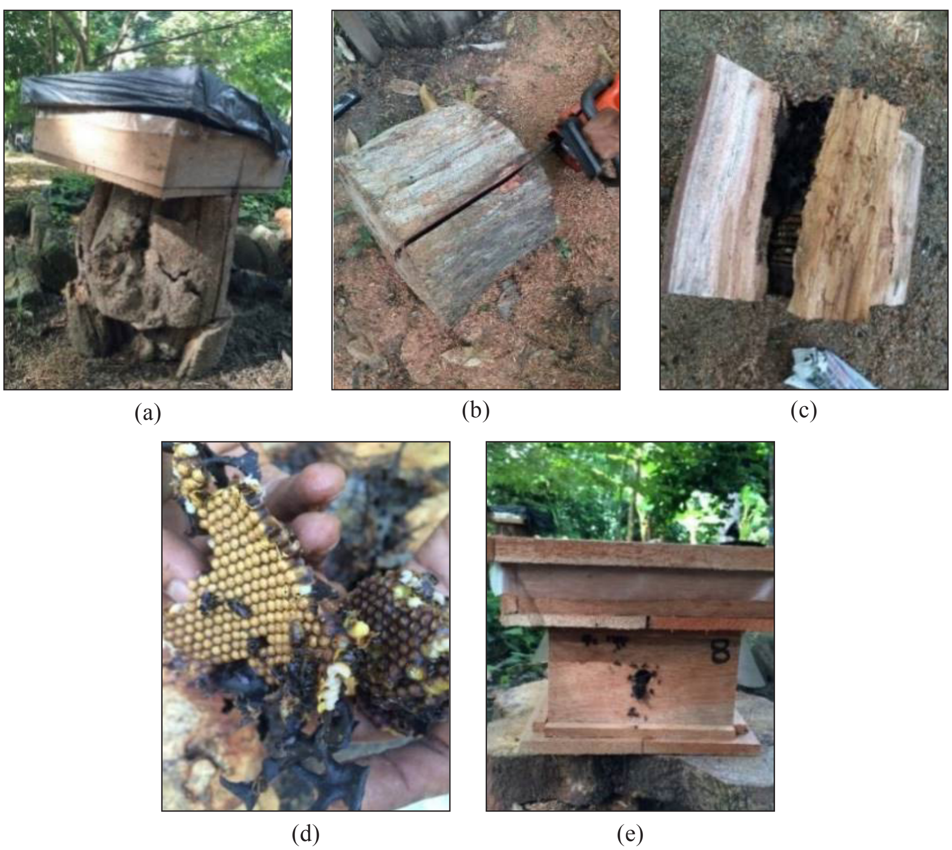

Figure 1. Colony transfer technique from logs to hives. (a) Log was cut and placed at designated farm. (b) Log was lined with a chainsaw. (c) After two weeks, log was twisted to expose the intact brood layers. The layers were transferred gently to a new hive. (d) Presence of egg-laying queen will ensure survivability of the colony. (e) The hive was placed at the previous location of the log

funnel entrance was sealed with clay at night and removed once the log had reached the designated farm. The log was left for at least two weeks to allow adaptation and repairing process by the bees. Next, the log was moved from the original location and lined using a chainsaw. The original location was replaced with a dummy box to avoid the foragers from being enraged of their missing nest. At the new location, the lined trunks were gently twisted to expose the intact brood layers which were then easily transferred to a new hive. This process was done meticulously to avoid destroying the queen or eggs. Presence of egg-laying queen will ensure survivability of the colony. Recovered honey and bee bread were removed to circumvent natural predators from attacking the fragile colony (Jaapar et al., 2016). The top space inside the hive was sealed with propolis to assist in colony repairing and survivability of the bees. Once done, the hive was placed at the previous location of the log (Figure 1). All colonies survived after one month of transfer to hives.

Colony Splitting Technique

Hives (*n*=10) were relocated from original location. The chamber was opened to expose the internal nest structure. Brood layers were removed and divided equally, i.e. the

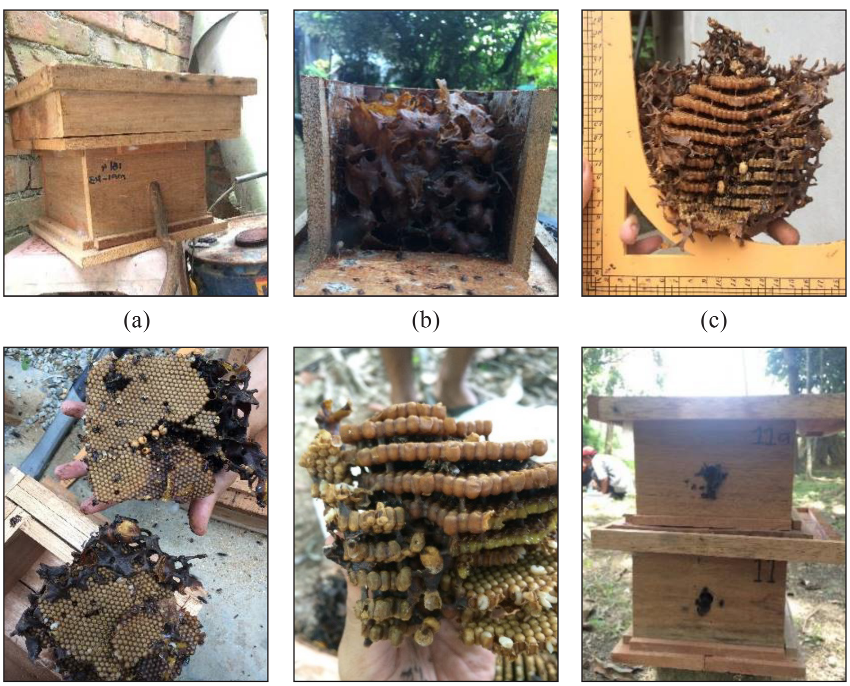

Figure 2. Colony splitting strategy from one hive into two hives. (a) Hive was relocated from original location. (b) The hive chamber was opened. (c) Brood layers were removed and (d) divided equally. (e) Presence of egg-laying queen or virgin queen cells will ensure survivability of the colony. (f) Both hives were returned to the original location and stacked, where the old hive containing mother colony was placed at the top of the new, daughter colony (new hive)

old hive contained half of the old and new broods with the presence of egg-laying queen (mother colony), while the new hive contained half of the new and old broods with the presence of a virgin queen cell (daughter colony). The division of brood layer, either horizontally or vertically, depending entirely on the location of the new and old broods. Then, both hives were returned to the original location and stacked, where the old hive containing mother colony was placed at the top of the new, daughter colony (Figure 2). All colonies could survive upon 12 weeks of observation. Parameters in height of brood cells, number of brood layer and number of queen cell were recorded immediately (week 0) and biweekly for 12 after colony splitting.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA, where each variable (height of brood cells, number of queen cell and number of brood layer) was analysed against one particular week. Correlation between the numbers of brood layer and queen cells was also performed. The data was transferred to graph pad Prism 7 for tabulation and analysis.

RESULTS AND DISCUSSION

Colony Transfer and Splitting Techniques

There are many ways to transfer or relocate a stingless bee colony, i.e. by colony expansion, drumming strategy or by using a dummy hive. In this study, the stingless bee colonies were transferred from logs to MUSTAFA-Hive. The bees, queen, broods, honey, bee bread and propolis were transferred immediately from the log after the inner structure of the log was exposed. The hive was closed and placed at the original location for colony repairing process by the bees. To facilitate the process, stingless bee honey was added in the hive regularly as extra food supplement for the bees. This technique requires less time for colony recovery compared to colony expansion or drumming strategies. A colony transfer is considered successful upon observation of new broods.

Colony splitting allows amplification of bee colonies which are reared in hives (Cortopassi-Laurino et al., 2006). The first well-documented colony splitting technique using stingless bee *Trigona carbonaria* reported an evenly split colonies, where the structure and the positioning of the brood chamber were well distributed (Heard, 1988). Amano (2004) showed colony splitting technique using stingless bees *T. carbonaria* and *Steatoda bipunctata*. The layer of the brood cells was divided equally and several large cells containing developing queen cells were scattered throughout the brood in the new colony. Compared to Amano (2004), our study recorded the number of queen cells distribution specifically and observation of these queen cell was made regularly. In addition, the position of the new and old hives was changed every week (top and bottom) to allow an equally active foraging activity of stingless bee worker and forager in both hives.

There were several parameters used to determine the readiness of colonies before splitting (data not shown); i) the minimum number of brood layers was more than ten, ii) there was a presence of egg-laying queen inside the colony, iii) the colony had actively performed the provisioning and oviposition process (POP), indicated by the full stocks of food and egg laying process by the queen, iv) the presence of a queen cell in the colony, and v) the broods inside the hive were full until they entered the honey cassette area. By placing the right amount of fully formed virgin queen cell inside the new hive, the chances of colony survivability will be high.

Nest Characteristics and Correlation between Numbers of Brood Layer and Queen Cell

Biweekly analysis of height of brood cells, number of brood layer and number of queen cells upon colony splitting were significantly different (P<0.05) except for height of brood on weeks 0 and 2 (P>0.05) (Table 1). The high value of standard deviation for numbers of brood layer and queen cell was due to the nature of the colony where the size and growth rate of each colony differs from one another. The height of brood cells increased over time upon colony splitting. For the first four weeks, the increment was slow as the workers were focusing in recovering the nest and ensuring enough food storage for the colony. This resulted in reduced number of brood layer during this stage. However, after four weeks, the colony has grown in its strength hence the gradual increase of height of brood cells, proportional to the number of brood layer, indicating a successful colony reconstruction.

Table 1
Biweekly analysis of height of brood cells, number of brood layer and number of queen cells upon colony splitting

Week	Parameters (mean ± SD)		
	Height of brood cells (cm)	Number of brood layer	Number of queen cell
0	14.5±1.20*	8.9± 2.13	5.2±1.32
2	14.5±1.22*	7.8±2.15	3.8±1.25
4	14.8± 1.19	6.7± 2.26	2.4±1.35
6	15.1± 1.13	8.5± 2.27	2.8±1.40
8	15.6±1.07	9.9± 2.23	4.0±1.76
10	15.9± 1.06	11.1±2.13	5.4±1.65
12	16.2± 1.05	12.1±1.85	6.4±1.65

Note. Analysis were performed to daughter colonies (*n*=10) and data were presented as mean ± standard deviation (SD). Significant differences among daughter colonies on a particular week (*), were determined by one-way ANOVA (P<0.05)

The number of queen cell in each colony which was reduced gradually within six weeks after colony splitting might reflect on the competency of workers. In honey bee *Apis mellifera*, it was found that workers regulate the queen rearing process by differentially constructing the cells (Hatch et al., 1999). After building different numbers of queen cells, over half (53%) of the constructed cells were destroyed by the workers themselves, in a non-random manner, prior to their emergence (Hatch et al., 1999). Therefore, a gradual increase of number of queen cells after six weeks, as shown by our study, indicating a positive gain of the workers aptitude towards colony survival and strength. The success of mother colonies were indicated by the presence of new queen cells, addition of brood layers and the formation of new broods by the queen. However, the data was not recorded in detail.

Despite a proportionally inclined (α) pattern, it was a weak correlation between numbers of brood layer and queen cells where the correlation coefficient (r=0.421), was very close to 0 (Figure 3). These data suggest that a high number of brood layer does not represent an increase in number of queen cells. Hence, the survival of a colony could not be determined based on number of brood layer.

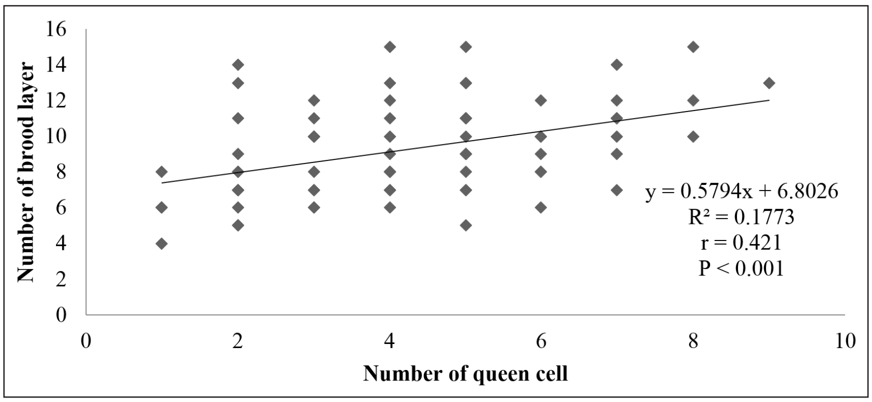

Figure 3. Correlation analysis between numbers of brood layer and queen cells

CONCLUSIONS

This study successfully described the colony transfer strategy from log to hive and the nest characteristics of height of brood cells, number of brood layer and number of queen cell for stingless bee *Heterotrigona itama* upon colony splitting. The colony transfer and splitting strategies proves to maintain survival of the daughter colony, which was indicated by the gradual increase of height of brood layer and number of queen cell. Judging from the weak correlation between numbers of brood layer and queen cell, further studies should consider the influence of workers in increasing the numbers of brood layer and queen cells for colony survival.

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CONFLICT OF INTEREST

None of the authors of this paper have any financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of this paper.

REFERENCES

Amano, K. (2004). *Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan*. Retrieved October 20, 2018, from http://www.fftc.agnet.org/library.php?func=view&id=20110913145638

Bakar, M. F. A., Sanusi, S. B., Bakar, F. I. A., Cong, O. J., & Mian, Z. (2017). Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. *Pakistan Journal of Nutrition*, *16*(11), 888-894.

Carey, J. R. (2001). Insect biodemography. *Annual Review of Entomology*, *46*(1), 79-110.

Cortopassi-Laurino, M., Imperatriz-Fonseca, V., Roubik, D. W., Dollin, A., Heard, T., Aguilar, I., … Nogueira-Neto, P. (2006). Global meliponiculture: Challenges and opportunities. *Apidologie*, *37*(2), 275-292.

Halcroft, M. T., Spooner-Hart, R., Haigh, A. M., Heard, T. A., & Dollin, A. (2013). The Australian stingless bee industry: A follow-up survey, one decade on. *Journal of Apicultural Research*, *52*(2), 1-7.

Hatch, S., Tarpy, D. R., & Fletcher, D. J. C. (1999). Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. *Insectes Sociaux*, *46*(4), 372-377.

Haydak, M. H. (1970). Honey bee nutrition. *Annual Review of Entomology*, *15*(1), 143-156.

Heard, T. A. (1988). Propagation of hives of *Trigona carbonaria* SMITH (Hymenoptera: Apidae). *Australian Journal of Entomology*, *27*(4), 303-304.

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annual Review of Entomology*, *44*(1), 183-206.

Jaapar, M. F., Jajuli, R., & Mispan, M. R. (2016). *Lebah kelulut Malaysia: Biologi dan penternakan* [Stingles bee of Malaysia: Biology and rearing]. Kuala Lumpur, Malaysia: Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI).

Jaffé, R., Pope, N., Carvalho, A. T., Maia, U. M., Blochtein, B., de Carvalho, C. A. L., … Imperatriz-Fonseca, V. C. (2015). Bees for development: Brazilian survey reveals how to optimize stingless beekeeping. *PLoS ONE*, *10*(3), e0121157.

Jalil, A. H., & Roubik, D. W. (2017). *Handbook of meliponiculture - Indo Malayan stingless bees clade*. Bangi, Malaysia: Akademi Kelulut Malaysia.

Kärcher, M. H., Menezes, C., Alves, D. A., Beveridge, O. S., Imperatriz-Fonseca, V. L., & Ratnieks, F. L. (2013). Factors influencing survival duration and choice of virgin queens in the stingless bee *Melipona quadrifasciata. Naturwissenschaften*, *100*(6), 571-580.

Kelly, N., Farisya, M. S. N., Kumara, T. K., & Marcela, P. (2014). Species diversity and external nest characteristics of stingless bees in meliponiculture. *Pertanika Journal of Tropical Agricultural Science*, *37*(3), 293–298.

Moo-Valle, H., Quezada-Euán, J. J. G., Canto-Martin, J., & González-Acereto, J. (2004). Caste ontogeny and the distribution of reproductive cells on the combs of *Melipona beecheii* (Apidae: Meliponini). *Apidologie*, *35*(6), 587-594.

Mustafa, M. Z., Yaacob, N. S., & Sulaiman, S. A. (2018). Reinventing the honey industry: Opportunities of the stingless bee. *Malaysian Journal of Medical Sciences*, *25*(4), 1-5.

Quezada-Euán, J. J. G., de Jesús May-Itzá, W., & González-Acereto, J. A. (2001). Meliponiculture in México: Problems and perspective for development. *Bee World*, *82*(4), 160-167.

Ranneh, Y., Ali, F., Zarei, M., Akim, A. M., Hamid, H. A., & Khazaai, H. (2018). Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *LWT-Food Science and Technology*, *89*, 1-9.

Saufi, N. F. M., & Thevan, K. (2015). Characterization of nest structure and foraging activity of stingless bee, *Geniotrigona thoracica* (Hymenopetra: Apidae; Meliponini). *Jurnal Teknologi*, 77(33), 69-74.

Shackleton, K., Al Toufailia, H., Balfour, N. J., Nascimento, F. S., Alves, D. A., & Ratnieks, F. L. (2015). Appetite for self-destruction: Suicidal biting as a nest defense strategy in *Trigona* stingless bees. *Behavioral Ecology and Sociobiology*, *69*(2), 273-281.

Singh, R. P. (2013). Domestication of *Trigona iridipennis* Smith in a newly designed hive. *National Academy Science Letters*, *4*(36), 367-371.

Sommeijer, M. J. (1999). Beekeeping with stingless bees: A new type of hive. *Bee World*, *80*(2), 70-79.

Tuksitha, L., Chen, Y. L. S., Chen, Y. L., Wong, K. Y., & Peng, C. C. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia-Pacific Entomology*, *21*(2), 563-570.