Flight Activities and Pollen Load of Three Species of Stingless Bees (Apidae: Melliponinae)

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Abstract. Stingless bees (Apidae: Melliponinae) are eusocial bees that have caste division within colony, namely queen, males, and workers. In the world, there are about 700 species of stingless bees and at least, 32 species have been described. In this study, we studied flight activities (returning and leaving of the nest) and pollen load of three species of stingless bees, i.e., Tetragonula laeviceps, Heterotrigona itama, and Lepidotrogona terminata. Morphologically, H. itama have a largest body size (average body length 5.52 mm), followed by L. terminata (average body length 4.99 mm), and T. laeviceps (average body length 3.86 mm). Peak activity of leaving and returning of the nest of H. itama occurred earlier (09.00-10.00) than in T. laeviceps and L. terminata (10.00-11.00). The highest pollen load occurred in L. terminata (270,950 pollen grains), followed by H. itama (69,802 pollen grains), and T. laeviceps (40,802 pollen grains). T. laeviceps carried dominantly pollen of banana flowers (Musa sapientum), L. terminata carried pollen of Acorus gramineus (Araceae), and H. itama carried pollen of Acanthaceae plant.

Key words: stingless bees, Tetragonula laeviceps, Heterotrigona itama, Lepidotrogona terminata, pollen load.

1. Introduction
In the world, they are approximately 700 species of stingless bees (Hymenoptera: Apidae). These bees are small (2-14 mm in size) and adapt very well in the tropic and subtropic areas [1]. Trigona (Tetragonula) is one of the genus of stingless bees belonging to the tribe Meliponini. Currently, 32 species of stingless bees were described [2], such as Trigona itama, T. thoracica, T. apicalis, T. terminata, T. respani, T. melanocephala, T. valdezi, T. collina, T. atripes, T. canifrons, T. iridepennis, and T. rufibasalia. Stingless bee is a high-level eusocial bee. One colony of T. laeviceps consists of up to 3000 worker, hundreds of drones, and one (or some) individual of queen. The task of worker bees including build nests, looking for nectar and pollen, resins or propolis, and keeping nest from predators [3]. The nest of stingless bees is commonly found in holes in stem or tree branches, in soil cavity, rock cavity, house-wall cavity, and wooden house.

Stingless bees are social insect that needs a lot of pollen and nectar for its colonies. By large numbers of worker bee individuals, this bee is potential as pollinator of various plants in the forest [4]. Stingless bee is also a good candidate for plants pollination in agricultural lands [1,5]. The study of the effect of pollination services by bees on agricultural land have been reported by researcher, previously. In cai son (Brassica rapa) plants in the edge of Gunung Halimun National Park, Trigona is one of the
genus that important to pollination process [6]. In agricultural crops in Malaysia, such as, mango, starfruit, durian, rambutan, and watermelon were visited by T. thoracica, T. apicalis, T. atripes, T. itama, and T. peninsularis. At Templer Park Forest Reserve and Selangor Fruit Valley, Malaysia also were reported six species of stingless bees, namely T. itama, T. thoracica, T. atripes, T. peninsularis, T. pagdeniformis, and T. Canifrons and dominant species were T. itama and T. thoracica. Four species (T. itama, T. thoracica, T. atripes and T. peninsularis) are potential for pollination in agricultural land [7].

Stingless bees are active throughout the year and as generalist insects that collect nectar and pollen from various plants species [8]. Research on the visiting activities its effectiveness of stingless bees has been widely reported. Harahap [9] reported highest activities of Trigona on strawberry was in 13:30-14:00. In teak plants (Tectona grandis), the peak activity of T. collina occurred in 10:00-12:00 [10]. Visiting activities of stingless bee on flowers also was affected by other species, especially with large body size. In this paper, we studied foraging activity (returning and leaving of the nest) and measured pollen load on three species of stingless bees (Tetragonula laeviceps, Heterotrigona itama, and Lepidotrogona terminata).

2. Materials and methods

2.1 Observation of Flight Activities

One colony of each species of stingless bees (Tetragonula laeviceps, Heterotrigona itama, and Lepidotrogona terminata) was used to observe the flight activities. The colonies were the collection of laboratory of Animal Biosystematics and Ecology, Department of Biology, Bogor Agricultural University. Flight activities recorded were returning and leaving of the nest. Flight activities were observed in 5 minutes in every hour from the morning until evening (07:00 – 17:00) using handycam. Observations of flight activity of each species were conducted in 15 days.

2.2 Observation of Pollen Load

Acetolysis method was used to observe the pollen load on stingless bees. One individual of worker bee (with pollen attached on hind legs) was put into tube contained 0.5 mL of ethanol:glycerol (4:1). The solution then rotated for 24 hours. After that, individual of bee was removed and was centrifuged at 2000 rpm for 10 minutes. Supernatant was discarded and remained as much as 0.1 mL. Remained solution was homogenized and dropped into Neubauer-type hemocytometer. The number of pollens were counted under compound microscope (400x10 magnification). The procedure was replicated as many as 20 individuals of each species. Image Raster 3 software was used to observe and measure of the pollens.

2.3 Data Analysis

The number of pollen load on body of each species of stingless bee was compared and analysed by using analysis of variance (One-Way ANOVA) and strengthened by Tukey test using Paleontological Statistics (PAST) software [11].

3. Results

3.1 Flight Activities

Observation of flight activities of three species of stingless bees were conducted with following weather conditions: the average air temperature 26-37°C, air humidity 42-81%, and light intensity 500-4800 lux (table 1).

Returning and leaving activities of T. laeviceps at 07:00 was 25 individuals/5 minutes) and increased until 12:00 (25-30 individuals/5 minutes). The activities decreased at 13:00-16:00 (20-25 individuals/5 minutes) and in the evening (17:00), the flight activity was very low (20 individuals/5 minutes) (figure 1).
Table 1. Environmental factors around hives of stingless bees observed in this study.

| Environmental conditions | Time       | Temperature (°C) | Humidity (%) | Light intensity (x 100 lux) |
|--------------------------|------------|------------------|--------------|----------------------------|
|                          | 07.00-08.00| 08.00-09.00      | 09.00-10.00  | 10.00-11.00                 |
| Temperature Average      | 26.41      | 27.46            | 32.16        | 35.89                      |
| Minimum                  | 24.52      | 25.6             | 23.99        | 30.08                      |
| Maximum                  | 28.77      | 29.68            | 33.33        | 43.22                      |
| Humidity Average         | 81.62      | 78.89            | 67.63        | 59.68                      |
| Minimum                  | 77.64      | 64.33            | 43.66        | 25.06                      |
| Maximum                  | 86.90      | 90.85            | 84.79        | 81.79                      |
| Light intensity Average  | 12.53      | 15.95            | 22.37        | 21.84                      |
| Minimum                  | 2.06       | 6.00             | 12.00        | 6.33                       |
| Maximum                  | 19.34      | 34.73            | 73.55        | 87.32                      |

Flight activity of *L. Terminata* was similar with *T. laeviceps*. In the morning (07:00), flight activity was very low (8 individuals/5 minutes) and peak activity occurred at 10:00 (40 individuals/5 minutes). The flight activities was still high at 11:00-15:00 (25 individuals/5 minutes). In the evening (17:00), flight activity was low (10 individuals/5 minutes) (figure 2).

Figure 1. Flight activities of *T. laeviceps* workers: activities of returning to the nest, activities of leaving of the nest.

Flight activity of *H. itama* was highest compared to others colony observed (*T. laeviceps* and *L. terminata*). The highest activities of *H. itama* occurred at 09:00 - 10:00 (90 individuals/5 minutes). Then, the activity decreased until 13:00-14:00 (50 individuals/5 minutes). In the afternoon (02:00-05:00 pm), the activity was still as high (60 individuals/5 minutes) (figure 3).

3.2 Pollen Load on Stingless Bees

Based on the data of measuring of pollen load of three species of stingless bees, *L. terminata* has a highest pollen load (270 950 pollen grains), followed by *H. itama* (69 802 pollen grains), and *T. laeviceps* (40 802 pollen grains). Pollen load of *L. terminata* was highest and different significantly with *H. itama* (p=0.0054) and *T. laeviceps* (p= 0.0013). There was no different significant between
pollen load on *H. itama* and *T. laeviceps* (p=0.886) (figure 4). Results indicated, each species of stingless bee showed the preferences to visit different flower plants. *T. laeviceps* prefer to visit banana flowers (*Musa sapientum*), *L. terminta* prefer to visit *Acorus gramineus* flowers (Araceae), while *H. itama* prefer to visit Acanthaceae plants.

Figure 2. Flight activities of *L. terminata* workers: — activities of returning to the nest, — activities of leaving of the nest.

Figure 3. Flight activities of *H. itama* workers: — activities of returning to the nest, — activities of leaving of the nest.

4. Discussion
Worker of *T. laeviceps* has a smallest body size (3.86 mm body length) compared to *L. terminata* (4.99 mm body length) and *H. itama* (5.52 mm body length). Body size of *T. laeviceps* measured in this study is similar with the same species reported in Sulawesi (3.40-3.43 mm body length) [12], in Asia and Sri Lanka (4.0-4.6 mm body length) [13], and in Singapore (3.50 mm body length) [14, 15].
Similarly, body size of *H. itama* (5.52 mm body length) is similar with the same species (7 mm) described by Lestari [16]. Meanwhile, body size of *L. terminata* measured in this study (4.99 mm body length) is slightly smaller than the description of the same species (6.15 mm body length) by Lestari [16].

![Figure 4. Pollen load on the body of stingless bees: H. itama, L. terminata, and T. Laeviceps](image)

Flight activity of *T. laeviceps* was high (25 individuals/5 minutes) in the morning (07:00) and peak activity occurred at 11:00 (25-30 individuals/5 minutes). In *L. Terminata*, peak activity occurred at 10:00 (40 individuals/5 minutes). The activities generally decreased at 13:00-16:00. Similar peak activity of *T. Laeviceps* (at 11:00) also was reported by Wulandari [17] in kailan plants (*Brassica oleracea* var Alboglabra) and the activity decreased in the afternoon. Hasibuan [18] also reported peak activity of *Tetrarogonula* sp. occurred at 12:00 -13.00 (10 individuals/minute, returning, and 9 individuals/minute, leaving the nest). Other study, Stein & Hensen [19] showed *Trigona* actively visit *Heconia angusta* flowers in 09:00-13:00. Inoue et al. [20] also reported *Trigona* active collect pollens in the morning related to its abundance. Stingless bees carried pollens on the corbicula located in the second tibia of the legs.

Peak activity of *H. itama* occurred earlier, i.e., at 09.00-10.00 (90 individuals/5 minutes) and the activities decreased in the afternoon. High activity in the morning (07.00-11.00) of *H. itama* also was reported by Lestari [16] to collect pollens and nectar. Foraging activities of stingless bees also related to weather conditions, such as air temperature, wind speed, rainfall, humidity, and light intensity [21]. Air temperature was positively correlated with daily activities of *T. itama* [22].

The highest pollen load (270 950 pollen grains) was carried by *L. terminata*, followed by *H. itama* (69 802 pollen grains), and *T. laeviceps* (40 802 pollen grains). The number of pollen grains carried by *L. terminata* was highest and significantly different with *H. itama* and *T. laeviceps*. Other study showed that large body size of stingless bees loaded by high number of pollens. Pangestika [23] reported highest pollen load occurred *H. itama* (31 392 pollen grains), followed by *L. terminata* (23 017 pollen grains), and *T. laeviceps* (8,015 pollen grains). Asmini [24] also reported highest pollen load occurred *H. itama* (36 650 pollen grains), followed by *L. terminata* (26 940 pollen grains), and *T. laeviceps* (9 700 pollen grains). In a kalean crop, Wulandari [17] reported the number of pollen carried by an individual of *T. laeviceps* was 8 125 pollen grains, while Hasibuan [18] reported *Tetrarogonula* sp. carried 19 476 pollen grains.

During foraging time, bees tend to visit the flowers of the same species of plant [25]. Results showed that *T. laeviceps* prefer to visit banana flowers (*Musa sapientum*), *L. terminata* visit the *Acorus*
gramineus flowers (Araceae), while *H. itama* visit Acanthaceae flowers. The composition of pollen carried by each species of stingless bee depend on the availability of plants around beehive. Hasibuana [18] reported *Tetragonula* sp. was loaded by 14 type of pollens, i.e., pollen of *Caesalpinia pulcherrima, Carica papaya, Cocos nucifera, Portulacaceae, Sapindaceae, Compositae, Chlorantaceae, Pinaceae, Solanaceae, Liliaceae, Umbelliferae, Polygonaceae-1, Polygonaceae-2, and Leguminosae.*

5. Conclusion

Body size of stingless bees observed varied. *Heterotrigona itama* has the largest body size (5.52 mm body length), followed by *L. terminata* (4.99 mm body length), and *T. laeviceps* (of 3.86 mm body length). Peak activity of *H. itama* occurred at 09.00-10.00, while in *T. laeviceps* and *L. terminata* occurred at 10.00-11.00. The highest pollen load occurred in *L. terminata* (270 950 pollen grains), followed by *H. itama* (69 802 pollen grains), and *T. laeviceps* (40 802 pollen grains). Plants species dominantly visited by a stingless bees were banana (*Musa sapientum*), *Acorus gramineus* (Araceae), and Acanthaceae plants.

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