Flight Activities and Pollen Load of *Lepidotrigona terminata* Smith (Apidae: Meliponinae)

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1. Introduction

Stingless bees (Apidae: Meliponinae) are eusocial insects that live in colonies (Michener 1974; Inoue et al. 1985). Stingless bees have caste division, consist of queens, males, and workers (Michener 1974). Queen bee has an important role as new individual producer, while drones mate the queen bee (Sakagami and Yamane 1987; Boongird and Michener 2010). The worker bees are the females that responsible for foraging, cleaning, guarding, and making a nest (Inoue et al. 1985; Sakagami and Yamane 1987; De Bruijn and Sommeijer 1997; Nugroho and Soesilohadi 2015).

Foraging of worker bees is an essential activity for collecting resources (pollen, nectar, resin, and wax) (De Bruijn and Sommeijer 1997; Boongird and Michener 2010). Nectar is a flower essence which from liquid containing sugar as an energy source of bees (Inoue et al. 1985; De Bruijn and Sommeijer 1997; Ramalho et al. 1998; Boongird and Michener 2010). Stingless bee need pollen and nectar as nutrition for individual and their colony (Ramalho et al. 1998; Atmowidi et al. 2007, 2008). However, Eltz and Gorke (2002) reported that, *Trigona collina* is not only used pollen and nectar as food sources but also fungal spores as alternative. Wax is a compound produced by plants used as substance for construction of bee’s nest (Wille 1983). Flight activity of worker bees also collect the resins. Resin is a main substance for nest construction used as defense system to against predators (Sakagami et al. 1989; De Bruijn and Sommeijer 1997; Lehmbreg et al. 2008; Duangphakdee et al. 2009).

Flight activities of stingless bees are influenced by several environmental conditions, i.e., temperature, light intensity, humidity, and wind velocity (Heard and Hendrikz 1993; Vicens and Bosch 2000; Nugroho and Soesilohadi 2015). Other factors such as food sources also could influence flight activities (Inoue et al. 1985; De Bruijn and Sommeijer 1997; Ramalho et al. 1998; Boongird and Michener 2010). Stingless bee need pollen and nectar as nutrition for individual and their colony (Ramalho et al. 1998; Atmowidi et al. 2007, 2008). However, Eltz and Gorke (2002) reported that, *Trigona collina* is not only used pollen and nectar as food sources but also fungal spores as alternative. Wax is a compound produced by plants used as substance for construction of bee’s nest (Wille 1983). Flight activity of worker bees also collect the resins. Resin is a main substance for nest construction used as defense system to against predators (Sakagami et al. 1989; De Bruijn and Sommeijer 1997; Lehmbreg et al. 2008; Duangphakdee et al. 2009).

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et al. 1985; Atmowidi et al. 2008; Amzi et al. 2015; Nugroho and Soesilohadi 2015). Consequently, study of flight activities (leaving and returning to the nest) and pollen load is an essential knowledge for pollination of stingless bee species. One of stingless bee species in Indonesia that has potential for pollination is *Lepidotrigona terminata* (Klein et al. 2003a, 2003b; Asmini 2016).

*Lepidotrigona terminata* is widely distributed stingless bee in Indonesia, including Java, Sumatera, Borneo, and Sulawesi (Klein et al. 2003a, 2003b; Smith 2012; Syafrizal et al. 2014). The species was characterized by yellow or white ring on the thoracic, in outside of mesocutum and mesoscutellum (Schwarz 1939; Smith 2012). Activities of *L. terminata* are effective for pollination, i.e., able to collect 33 pollen types in Thailand (Jongjitvimol and Poolprasert 2014), increases fruit formation in coffea (Klein et al. 2003a, 2003b), and enhance number of seeds in mustard (Asmini 2016). Moreover, they could enhance pollination because its small body size which compatible with small flowers (Amzi et al. 2015). Unfortunately, study of flight activities and pollen load of *L. terminata* rarely conducted. Therefore, this study aimed to i) measure the flight activities of *L. terminata*, ii) counted and identified carrying pollen load, and iii) examined the correlation between flight activities and environmental conditions.

2. Materials and Methods

2.1. Study Sites

Observation of flight activities for one colony *L. terminata* was conducted from August to December 2016 in Field Laboratory of Animal Biosystematics and Ecology, Bogor. In around of the bee colony were found wild plants such as trees, weeds, and flowering plants. Pollen type and pollen load were identified and measured at laboratory of Animal Biosystematics and Ecology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java.

2.2. Procedures

2.2.1. Observation of Flight Activities

One colony of *L. terminata* was used to observe the flight activities. Size of the bee colonies of *L. terminata* used was relatively large (2,000-2,500 individuals). Observation of flight activities (leaving and returning to the nest) were conducted one minute in each one hour interval starting from 07.00 to 17.00 for 15 days in a sunny day. Those activities were recorded by using handycam Panasonic (60x zoom). The returning number of individual with carrying pollen and resin, and the leaving number of individual with and without carrying garbage were counted (Figure 1). Individuals of *L. terminata* that did not carry pollen or resin were assumed to carry nectar. Measurement of environmental factors (temperature, humidity, and light intensity) were conducted before observation of flight activities.

2.2.2. Pollen Identification

The pollen identification was used by acetolysis method (Erdtman 1960). Fifteen individuals of *L. terminata* that returning to the nest with pollen load were collected using insect net. Pollens on the legs were taken by using sterile tweezers, and then, those pollens were put in a 1.5 ml tube with 1 ml acetolysis solution. Furthermore, there were heated at 80°C in water bath for 5 minutes and were centrifuged at 3,500 rpm for 10 minutes. Acetolysis solution was discarded from tube while pollen that kept in the tube was rinsed with distilled water and were continuously centrifugated at

Figure 1. Activity of leaving and returning to the nest of *L. terminata*, (a) returning to the nest carrying pollen, (b) returning to the nest carrying resins, (c) returning to the nest carrying nectar, and (d) leaving the nest with and without carrying garbage. The arrows represent activity of each bee individuals
3,500 rpm for 10 minutes. Rinsing was repeated until the rinse solution looked clear. Pollens were dried in oven at 60°C for 24 hours. After that, 0.5 ml of glycerin 30% was added and tube was stirred until there was no clot. The pollens were prepared on the slides using micropipette and was covered. Then, the pollens were observed under a Nikkon C-LEDS 102 microscope (30x magnification) and were captured using optilab camera. All pollens were identified based on morphological characters i.e., the numbers of apertures, pollen shape (polar and equatorial view), ornaments of exine, and pollen size (Huang 1972; APSA 2007).

2.2.3. Pollen Load Measurement

Pollens from 15 worker bees were collected. Each individual of worker bees with pollen on hind legs was put into the tube contained 0.5 ml of alcohol:glycerol (4:1). Then rotated for 24 hours and individual was removed from tube. The tube was centrifuged at 2,000 rpm for 10 minutes. The supernatant was discarded and remained as much as 0.1 ml with sediment. Remaining solution was homogenized and the solution was dropped into Neubauer-type hemocytometer. The numbers of pollens were counted by using compound microscope (400 x 10 magnification). Image Raster 3 software also was used to observe and measure the pollens.

2.3. Data Analysis

Correlation between flight activities of L. terminata and environmental factors (temperature, humidity, and light intensity) were analyzed by Pearson correlation using Minitab 16.0 software. The selection resource of individual L. terminata was analyzed using modification formula of 1) selection index (\(w_i\)), 2) G-test (Chi-squared) (\(x^2\)), and 3) comparison of two selection index (\(x^2\)) (Manly et al. 1993; Krebs 1998). Those modification formulas were showed as below:

1. \[ w_i = \frac{o_i}{p_i} \]
2. \[ x^2 = 2 \sum_{i=0}^{n} \left[ u_i \ln \left( \frac{u_i}{U_{pi}} \right) \right] \]
3. \[ x^2 = \frac{(\bar{w}_i - \bar{w}_j)^2}{\text{variance} (\bar{w}_i - \bar{w}_j)} \]

Where, \(w_i\) and \(w_j\) representing the forage ratio of number individuals for resource \(i\) or \(j\), \(o_i\) and \(o_j\) representing the proportion of number pollen of resource \(i\) or \(j\), \(p_i\) and \(p_j\) representing the proportion of number individuals carrying pollen types \(i\) or \(j\), \(u_i\) and \(U\) representing the number and total number of individual observations, respectively.

3. Results

3.1. Flight Activities of L. terminata

The leaving and returning to the nest of L. terminata have similar pattern (Figure 2). The activities started in early morning 07.00-08.00 with low number of individuals (1 individual/min). The number of individuals gradually increased until 09.00-10.00 and there were relatively constant from 09.00-13.00. However, the peak flight activities occurred at 10.00 to 13.00 (8 and 6 individuals/min for leaving and returning to the nest). The flight activities were slightly decreased from 14.00-15.00 to 16.00-17.00 (2 individuals/min).

Activity of leaving the nest without carrying garbage was constantly higher than with carrying garbage (Figure 3a). The peak activities of leaving the nest carrying garbage occurred at 09.00-10.00 (2 individuals/min). Leaving activities without carrying garbage were relatively constant from 08.00-14.00. However, the peak activities occurred at 12.00-13.00 (8 individuals/min) and then slightly decreased. Meanwhile, the activities of returning to the nest showed various patterns (Figure 3b). Peak of activities in returning to the nest L. terminata carrying pollens occurred at 09.00-10.00 (2 individuals/min). Contrary, activities of carrying pollen decreased, whereas activities of carrying resins and nectar increased at 12.00-13.00. Peak activities carrying resins (1 individual/min) and nectar (5 individuals/min) were relatively constant from 09.00-15.00.

Environmental factors such as temperatures, humidity, and light intensity around the nest of L. terminata varied during sampling period (Table 1). The average of minimum and maximum temperatures occurred at 07.00-08.00 (26.2°C) and at 12.00-13.00 (37°C), respectively. Contrary, average of minimum and maximum humidities presented at 12.00-13.00 (39.3%) and at 08.00-09.00 (81.1%), respectively. The average of minimum light
3.2. Pollen Types and Pollen Load

Based on morphological characters of pollen carried by L. terminata, pollen was classified into four types of plant family. Those pollens belong to Anacardiaceae, Araceae, Aceraceae, and Acanthaceae (Figure 4). Pollen type of Anacardiaceae and Araceae have a small size with approximately 10 µm in diameter. While, pollen type of Aceraceae and Acanthaceae have a large size with approximately 50 µm in diameter.

The average of pollen load of L. terminata was 32,696 pollen grains (Table 3). The highest number of pollen grains of Araceae mostly attached in hindlegs of L. terminata (452,405 pollen grains). Meanwhile, the lowest number belong to Acanthaceae (1,445 pollen grains).

The highest of selection index was Araceae ($\bar{x}_i=1.522$), followed by Anacardiaceae ($\bar{x}_i=0.242$), Aceraceae ($\bar{x}_i=0.032$), and Acanthaceae ($\bar{x}_i=0.003$) (Table 3). There were significant different of selection index among pollen types ($x^2=39.32$, $p<0.01$). Based on two selection indices test, pollen of Araceae was significantly differences with Anacardiaceae, Aceraceae, and Acanthaceae ($x^2 >83$, $p<0.01$).
Meanwhile, there were not significantly differences between pollen of Anacardiaceae with Aceraceae and Acanthaceae ($x^2 < 1.015$, $p > 0.05$). Moreover, no significant different was also found between Aceraceae and Acanthaceae ($x^2 = 1.027$, $p > 0.05$).

Figure 3. Activity of leaving and returning to the nest L. terminata, (a) leaving with and without carrying garbage and (b) returning carrying nectar, pollens, and resins.
Table 1. Environmental factors (temperature, humidity, and light intensity) measured around the nest L. terminata during observations

| Parameters          | 07.00-08.00 | 08.00-09.00 | 09.00-10.00 | 10.00-11.00 | 11.00-12.00 | 12.00-13.00 | 13.00-14.00 | 14.00-15.00 | 15.00-16.00 | 16.00-17.00 |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Temperature (°C)    |             |             |             |             |             |             |             |             |             |             |
| Average             | 26.2        | 27.2        | 29.8        | 32.5        | 36.7        | 37.0        | 36.0        | 33.6        | 31.6        | 28.6        |
| Minimum             | 24.5        | 25.3        | 23.9        | 29.4        | 31.0        | 31.8        | 26.0        | 25.8        | 25.4        | 24.6        |
| Maximum             | 27.3        | 29.2        | 33.3        | 37.3        | 42.6        | 43.1        | 43.7        | 40.8        | 39.3        | 32.6        |
| Humidity (%)        |             |             |             |             |             |             |             |             |             |             |
| Average             | 81.1        | 78.2        | 64.9        | 56.8        | 42.8        | 39.3        | 42.9        | 52.2        | 58.8        | 68.2        |
| Minimum             | 77.0        | 64.0        | 43.0        | 33.0        | 25.0        | 24.0        | 22.0        | 26.0        | 31.0        | 45.0        |
| Maximum             | 86.0        | 89.0        | 71.0        | 73.0        | 72.0        | 57.0        | 79.0        | 87.0        | 81.0        | 88.0        |
| Light intensity (x 100 lux) |     |             |             |             |             |             |             |             |             |             |
| Average             | 12.7        | 15.7        | 18.9        | 20.9        | 21.7        | 25.5        | 21.3        | 17.7        | 5.9         | 4.2         |
| Minimum             | 2.0         | 8.0         | 12.0        | 6.0         | 11.0        | 10.0        | 3.0         | 1.0         | 1.0         | 1.0         |
| Maximum             | 19.0        | 34.0        | 31.0        | 55.0        | 43.0        | 51.0        | 90.0        | 54.0        | 13.0        | 10.0        |

Table 2. Pearson correlation (r) and probability (p) between environmental factors and flight activities

| Parameters     | Returning carrying nectar | Returning carrying pollens | Return carrying resins | Leave without carrying garbage | Leave carrying garbage |
|----------------|---------------------------|----------------------------|------------------------|--------------------------------|------------------------|
| Temperature    | r=0.768, p=0.009          | r=0.581, p=0.600          | r=0.573, p=0.611      | r=0.741, p=0.015              | r=0.195, p=0.590      |
| Humidity       | r=0.760, p=0.011          | r=0.813, p=0.004          | r=0.578, p=0.631      | r=0.720, p=0.019              | r=0.195, p=0.590      |
| Light intensity| r=0.841, p=0.002          | r=0.678, p=0.018          | r=0.573, p=0.611      | r=0.884, p=0.018              | r=0.548, p=0.101      |

Figure 4. Images of various pollen types attached on L. terminata, (a) polar and (b) equatorial view, 1: Anacardiaceae, 2: Araceae, 3: Aceraceae, and 4: Acanthaceae
4. Discussion

4.1. Flight Activities

Based on Pearson correlation test, environmental factors correlated with flight activities (Table 2). In lower temperature, bees increase thoracic temperature with wings flapping (Stone and Willmer 1989). Bees adjust to the ambient temperature with their thermoregulation system (Eltz et al. 2001; Syafri zal et al. 2014). Bees also use a light as direction to forage and increase body temperature. Limitation of light may to restrict flight activity of bees, like Megalopta genalis and Trigona carbonaria (Heard and Hendrikz 1993; Kelber et al. 2006). Air humidity also affected the activity of L. terminata where increasing humidity could decrease flight activity. Flight activities of leaving and returning to the nest increased in the morning to mid-day and decreased in the afternoon (Figure 2). When temperature was high in the mid-day (26.2-37°C), stingless bees are actively foraging (Stone and Willmer 1989; Nugroho and Soesilohadi 2015). Meanwhile, there was no activity of leaving and returning to the nest when raining.

Flight activities of returning to the nest carrying pollen occurred in the morning (09.00-10.00) (Figure 3b). Those, activity increased in average temperature 29.8°C and humidity 64.9% (Table 1). In the morning, there are much pollen in the flower (Sommeijer et al. 1983; Inoue et al. 1985; Atmowidi et al. 2008), whereas in the afternoon there are less pollens, however bees still need to visit some flowers to collect pollen (Atmowidi et al. 2007). Amzi et al. (2015) stated that time of flower anthesis occurs mostly in the morning and the flowers are close mostly in the afternoon. The pollen-taking process such as flight range, pollen collection and pollen handling time associated to flowers blooms that mostly in the morning. Flight range of bees are farther and need a lot of time for pollen collection (Inoue et al. 1985; Biesmeijer and Toth 1998). Time for pollen collection, pollen load and pollen quality also affect the flight activities of bees and productivity of the colony (De Bruijn and Sommeijer 1997; Biesmeijer and Toth 1998; Ramalho et al. 1998; Nagamitsu and Inoue 2002). The large

Table 3. Number of pollen from each individual of L. terminata and selection index ($w_i$) of each plant family

| Individual | Araceae | Anacardiaceae | Aceraceae | Acanthaceae | Total |
|------------|---------|---------------|-----------|-------------|-------|
| 1          | 99,275  | 0             | 0         | 0           | 99,275|
| 2          | 0       | 8,150         | 0         | 0           | 8,150 |
| 3          | 30,915  | 0             | 0         | 4,600       | 35,515|
| 4          | 0       | 5,525         | 0         | 11,055      | 16,580|
| 5          | 0       | 0             | 0         | 0           | 0     |
| 6          | 87,070  | 0             | 0         | 0           | 87,070|
| 7          | 5,235   | 0             | 0         | 0           | 5,235 |
| 8          | 0       | 7,260         | 0         | 0           | 7,260 |
| 9          | 85,885  | 0             | 0         | 0           | 85,885|
| 10         | 133,385 | 0             | 0         | 0           | 133,385|
| 11         | 5,920   | 0             | 0         | 0           | 5,920 |
| 12         | 0       | 0             | 0         | 1,445       | 1,445 |
| 13         | 3,260   | 0             | 0         | 0           | 3,260 |
| 14         | 1,460   | 0             | 0         | 0           | 1,460 |

Number of pollen | 452,405 | 20,935 | 15,655 | 1,445 | 490,440 |
Average of pollen | 30,160 | 1,395.6 | 1,043.6 | 96.3 | 32,696 |
Selection index ($w_i$) | 1.522 | 0.242 | 0.165 | 0.069 |
number of *L. terminata* individual carrying pollen in the morning indicated effective time for pollination where pollen foraging in mid-day until afternoon slightly decreased (Figure 3b). Other factors also affect foraging of bees such as landscapes structures where forest habitat is important element of landscape for bees because it provides diversity of plant resources (nectar, pollen, and resin) (Priawandiputra et al. 2015).

Peak of flight activity returning to the nest carrying resins occurred in the mid-day (11.00-13.00). Based on observations, activities of *L. terminata* carrying resin was performed in high ambient temperature 37°C (Figure 3). Sticky character of resin on the hind tibias of Melipona stingless bees disturbs flight activities (De Bruijn and Sommeijer 1997). Results showed that activity carrying resin from morning to afternoon was the lowest compared to other activities, such as carrying nectar and pollen. Based our observation and Inoue *et al.* (1985), individual of bee who carrying resin take longer handling time (23 minutes) and spend a longer time duration of staying in the nest. This suggested that the sticky character of resin caused a long handling time to release resin from the body.

Flight activities of bees forager could associate with choice for collecting material. Observation showed that activity of carrying resins and nectar increased when the activity of carrying pollen decreased (Figure 3b). This could be occurred due to task switching of bee foragers in collecting resources (pollen, nectar, and resin). The same individual foragers of *L. terminata* might switch from pollen—collecting to resin—collecting or nectar—collecting in a day. There are several bee foragers who perform to switch become pollen—nectar foragers (Inoue *et al.* 1985; De Bruijn and Sommeijer 1997; Biesmeijer and Toth 1998). Inoue *et al.* (1985) stated the pollen forager of stingless bees can switch to collect nectar and conversely, but they do not switch to take the resin on *T. minangkabau*. Collecting resin is a special task in Melipona due to limited number of bee foragers (Biesmeijer and Toth 1998). However, in present study, the pollen—collecting stingless bee could switch their job position into the resin—collecting where the handling time between pollen—collecting and resin—collecting are quite similar (De Bruijn and Sommeijer 1997). The choice for collecting material of stingless bee are influenced by colony needed (Biesmeijer and Toth 1998).

Flight activity of leaving the nest without carrying garbage were higher in the mid-day (12.00-13.00). Flight activities leaving the nest without carrying garbage at the same intervals were proportional with activity of returning to the nest carrying nectar and resin (Figure 3). It may be the individual bees who leaving the nest without carrying garbage was the same individuals of returning to the nest carrying nectar and resins. Leonhardt *et al.* (2007) reported that high activity of leaving the nest is largely take the nectar. Activity of leaving the nest with carrying garbage occurred in the morning (09.00-1000) (Figure 3a). Peak of activity leaving the nest with carrying garbage occurred in the same intervals with activity carrying pollen. Biesmeijer and Toth (1998) also reported that activity of carrying garbage occurred in the morning where bees flew around the nest (orientation flight) without collecting any materials. In the morning, throw out activity of garbage could be performed to clean the nest from the residue of egg hatched. It may be related to the eggs place formation for increasing the productivity of the colony.

### 4.2. Pollen Types and Pollen Load

Four types of pollens were identified in this study consists of four plant families (Figure 4). The abundance and proximity of food sources will be optimized in getting pollen and nectar. Moreover, the food availability, quality, and quantity of pollen and nectar in each flower caused stingless bee choose food source preference (Heard 1999; Jongjitvimol and Wattanachaiyingcharoen 2006). The dominant pollen carried by *L. terminata* was pollen of Araceae (Table 3). It was suggested high availability of Araceae in this area and it was preferred by *L. terminata*. Amzi *et al.* (2015) stated that fragrance and flower shape affect bees to find food. The previous report by Pangestika (2016) showed that four pollen types carried by *L. terminata* in West Java, Indonesia. It showed that, the pollen types collected by *L. terminata* from October to November were not significantly different with this research. In the other hand, pollen types carried by 10 colonies of *L. terminata* in longer observation there were 38 pollen types in the Nam Nao National Park Thailand (Jongjitvimol and Poolprasert 2014) and 11 pollen types in Terengganu, Malaysia (Amzi *et al.* 2015). It is showed that number of colonies, observation time, and landscape structure greatly influences the number of pollen types collected by the stingless bees (Ramalho *et al.* 1994; Priawandiputra *et al.* 2016).
The number of pollen types could be related to the availability and flowering period of plants as food sources. Generally, stingless bees have a preference for food source in the environment. Stingless bee species shows different food preferences (Eltz et al. 2001; Jongjitvimol and Wattanachaiyingcharoen 2006). Foraging activities of bees tend to visit flowers of the same plant species (flower constancy) (Table 3). On a single trip, bee commonly visit one species of plants. The visiting consistency of bee in collecting flower resources relates to the quality and quantity of pollen and nectar (Heard 1999).

The average of pollen load carried by individual of *L. terminata* was 32,696 pollen grains (Table 3). Meanwhile, other studies showed that same bee species carried less pollen grains compared to present study, i.e., 26,940 pollen grains (Asmini 2016) and 23,017 pollen grains (Pangestika 2016). The number of pollen grains carried by bees depends on the pollen type (Ramalho et al. 1994). Our results showed that *L. terminata* prefer pollen of Araceae because its small size (±10 µm), while other studies showed that most pollen attached in *L. terminata* was belonging to Euporbiaceae and Brassicaceae where the pollen sizes are more than 50 µm (Asmini 2016; Pangestika 2016). However, some individuals in present study also carried large pollen, such as pollen of family Anacardiaceae and Aceraceae (Figure 4). The various shape, size, colour, and odor of flowers are an important factors for food preference of bees (Amzi et al. 2016; Jongjitvimol and Wattanachaiyingcharoen 2006). *Lepidotrigona terminata* can handle small size flower to collect the pollen and nectar resources. Heard (1999) stated that activities of bees to collect the pollens related to plants pollination. These results indicated that *L. terminata* is one species of stingless bees that potentially for plants pollination.

In conclusion, this study has provided flight activities information which represent the effective foraging activity to collect pollen, nectar, and resin of *L. terminata*, from the morning to mid-day. During foraging period, *L. terminata* carried four pollen types where the dominant pollen was from Araceae (452,405 pollen grains). The size of pollen grains is one of the main factors in attracting the *L. terminata*. Identification of pollen type attached on *L. terminata*, is provides the important information about which plants as sources of bee forage and also to be used as cultivation strategy of bees. Some environmental factors, such as temperature, light intensity, and humidity could affect the flight activities of *L. terminata*.

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