Differences of the Daily Flight Activity Rhythm in Two Neotropical Stingless Bees (Hymenoptera, Apidae)

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Abstract

Stingless bees are mainly tropical and subtropical, eusocial bees and perform activities that are both internal and external to the nest. This study aims to investigate and compare the daily flight activities of *Melipona scutellaris* Latreille and *Frieseomelitta doederleini* (Friese). The daily flight activities of the two Meliponini species was regular for both initial and final activities and for preferential time of activity throughout the months, which may indicate the presence of a biological rhythm synchronized by the daily light-dark cycle. Temperature, light intensity and relative humidity probably influenced the rhythms of activity during the day, in a way that may act as a modulator of bee rhythms. *M. scutellaris* was the larger and darker bees and showed earlier activities and in lower temperatures when compared to *F. doederleini*, which were smaller bees.

Introduction

Bees from Meliponini tribe manifest elaborated social behaviour and are characterised by having perennial colonies, presenting morphological and behavioural differences in the female castes, and some workers forage on floral resources that are important for the colony, especially for feeding the offspring (Michener, 2000). These foraging activities may occur at specific times of the day, as has been observed in studies on flight activities of several stingless bee species (Hilário et al., 2003; Pierrot & Schlindwein, 2003; Fidalgo & Kleinert, 2007).

Flight activities may be related to a range of intrinsic and extrinsic factors. Among the intrinsic factors, the colony’s reproductive phase may be related to flight activity. Nunes-Silva et al. (2010) observed that *Plebeia remota* (Holmberg) foragers collected more pollen in those months that corresponded to the colony’s reproductive season, generally the summer, and collected more nectar in the reproductive diapause (the winter months).

Bee flight activities at specific times of the day may be manifestations of rhythms resulting from the endogenous biological clock, as reported in several insect studies (Koukkari & Sothern, 2006). Biological rhythms related to daily activity have been detected in *Scaptotrigona aff. depilis* (Moure) (Bellusci & Marques, 2001) and in *Frieseomelitta varia* (Lepetier) (Almeida, 2004; Oda et al., 2007).

Extrinsic factors, particularly abiotic factors (temperature, light intensity and relative humidity), may also modulate the daily flight activities of eusocial bees. Studies of the external daily activities of *Melipona asilvai* Moure (Souza et al., 2006) and *Plebeia pugnax* Moure (in litt.) (Hilário et al., 2001) have detected the effects of temperature leading to an increase and the effects of relative humidity leading to a decrease of bee activities. The interaction between the pattern of temperature change in the environment and the endothermic ability in bees has strong implications for when and where a particular bee can fly and thus to the resulting pattern of activity (Willmer & Stone, 2004). According to Heinrich (1993), ambient temperature in relation to certain bee morphology...
characteristics, such as colour and size, may, through the thermoregulatory mechanism, influence the timing of bee flight activity, so the smallest bees generally have a higher heat production rate per unit mass than the greatest bees.

The timing of eusocial bee activity to collect resources may also be influenced by the time at which this resource is available in the environment (Roubik, 1989).

In this work, we studied the differences in the daily rhythm of flight activity of *Melipona scutellaris* Latreille and *Frieseomelitta doederleini* (Friese), and the influence of environmental and meteorological factors. We also investigated the relationship between the daily rhythm and body size of these two stingless bees.

**Materials and Methods**

**Studied species**

In order to conduct the study, we selected two colonies of bees from Meliponini belonging to two different species: *M. scutellaris* and *F. doederleini*, located in an apiary in the village of Pedra Branca. Bees from the two colonies were captured in areas near the apiary, where they were kept in wooden boxes covered with asbestos shingle, for at least one year. Both nests were close to each other and on a wood shelf outdoors. The bees were not fed artificially during the study period.

To analyse bee body size, measurements were made of the thorax width (inter-regular width - distance between wing bases) in 10 forager individuals from each species. According to Cane (1987), this is the more reliable measure to consider bee body size. Bee size classification was based on the parameters of Roubik (1989) and Michener (2000) and was statistically tested by Analysis of Variance (ANOVA) and the Tukey-Kramer multiple comparison test.

Samples of the two Meliponini species were collected and deposited in the Johan Becker Entomological Collection of the Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS).

**Study area**

Pedra Branca (12°44'30"S, 39°34'50"W, at an altitude of 300 m) is located in Santa Terezinha town, in the foothills of the Jibóia mountain range (Bahia State, Brazil). The predominant vegetation in Pedra Branca is the opened arboreal Caatinga (dryland) with palm trees (SEI, 2012). The Santa Terezinha town presents climate transition from sub-humid and dry to semi-arid, with average annual temperature ranging between 19°C and 27°C and annual rainfall from 800 to 1100 mm (SEI, 2012).

Daily relative humidity and temperature data were collected with a digital Thermo Hygrometer and light intensity data was measured using a digital luximeter (Lutron LX-107) at a distance of 100 cm from the soil, at the entrance to the studied colonies and in the nearest open air area. During the study period, the daily temperature varied from 19 to 36°C (26.5 ± 2.9°C, mean ± SD). The highest average temperature was recorded between November and January 2010 (28 ± 3.3°C) and the lowest average temperature was recorded in March 2010 (25 ± 1.9°C). The relative humidity varied from 39 to 99% (67 ± 13.7%) and the light intensity in the open air area from <1 to 82,700 lux.

Sunrise and sunset times were obtained from the Brazilian National Observatory (http://euler.on.br/ephemeris/index.php). Time of sunrise varied between 05:01 h (November 2009) and 06:00 h (July 2009) and time of sunset varied between 17:20 h (May 2010) and 18:13 h (January 2010).

**Flight activity**

We quantified the flight activity of the colonies of the two species of studied bees by counting the bees that entered and exited the nest (without apparently carrying material), that entered with pollen, with resin on the corbiculae. Observations and activity counts were conducted over three consecutive days in seven months (July, August, September and November 2009 and in January, March and May, 2010), from 04:00 to 19:00 h, for fifteen minutes in each hour of observation for each bee colony.

For the two studied species, analyses of the daily activities external to the nest were carried out using the Rayleigh test of Circular Statistical Method (Batschelet, 1980). Preferential times for activities (or acrophases) were considered for values with the vector (r) significant above 0.7 (varying from 0 to 1, according to the dispersion of data) and p < 0.05. Analyses were only carried out for values (number of individual bees) above 10. The daily activities of both colonies were compared using the Watson-Williams test (p < 0.05) (Circular Statistical Method - Batschelet, 1980).

**Results**

The two Meliponini species had different average widths, 2.9 ± 0.11 mm for *M. scutellaris*, considered to be medium-small, and 1.3 ± 0.04 mm for *F. doederleini*, which is considered to be small (difference observed through ANOVA and Tukey-Kramer multiple comparison test, P < 0.05).

*Melipona scutellaris* and *F. doederleini* occupied all the space within the wooden boxes and had many pots of food. Additionally, in all the months of the study, the colonies of *M. scutellaris* and *F. doederleini* demonstrated a large number of total activity (entrance + exit) and entrance with pollen. In general, when compared to *F. doederleini*, *M. scutellaris* collected less resin (Table 1). *M. scutellaris* and *F. doederleini* presented a higher number for total activity and entrance activity with pollen in January and March 2010. *M. scutellaris* also had a high number for bee entrance with pollen in August.
Table 1. Number of bee entrances and bee exits (total activity) and entrance activity with pollen and entrance activity with resin for *Melipona scutellaris* and *Frieseomelitta doederleini*, in the village of Pedra Branca (Santa Terezinha, Bahia, Brazil).

<table>
<thead>
<tr>
<th>Months</th>
<th><em>Melipona scutellaris</em></th>
<th></th>
<th></th>
<th><em>Frieseomelitta doederleini</em></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(total)</td>
<td>(pollen)</td>
<td>(resin)</td>
<td>(total)</td>
<td>(pollen)</td>
<td>(resin)</td>
</tr>
<tr>
<td>July/09</td>
<td>3911</td>
<td>576</td>
<td>80</td>
<td>1231</td>
<td>196</td>
<td>565</td>
</tr>
<tr>
<td>Aug/09</td>
<td>3054</td>
<td>2144</td>
<td>4</td>
<td>846</td>
<td>188</td>
<td>748</td>
</tr>
<tr>
<td>Sept/09</td>
<td>3510</td>
<td>742</td>
<td>15</td>
<td>625</td>
<td>134</td>
<td>806</td>
</tr>
<tr>
<td>Nov/09</td>
<td>6545</td>
<td>844</td>
<td>84</td>
<td>995</td>
<td>176</td>
<td>1309</td>
</tr>
<tr>
<td>Jan/10</td>
<td>8804</td>
<td>1852</td>
<td>44</td>
<td>2115</td>
<td>508</td>
<td>2232</td>
</tr>
<tr>
<td>Mar/10</td>
<td>12053</td>
<td>1431</td>
<td>75</td>
<td>1713</td>
<td>598</td>
<td>1860</td>
</tr>
<tr>
<td>May/10</td>
<td>1626</td>
<td>92</td>
<td>20</td>
<td>241</td>
<td>13</td>
<td>359</td>
</tr>
</tbody>
</table>

2009 (Table 1).

*Melipona scutellaris* began their total daily flight activities (entrance + exit) and entrance activity with pollen between 04:00 (November) and 06:00 h (July) (Fig 1) coinciding with the time of sunrise, which occurred earlier in November and later in July. Furthermore, temperature values were higher in the former than in the later month. The initiation of total flight activities and entrance activity with pollen for *F. doederleini* occurred between 05:00 (November and January) and 08:00 h (July), being overall later than those of *M. scutellaris*. (Fig 1).

Overall, throughout the study months, the temperature during the first flight activities of the two bee species varied from 19 to 26°C (average: 23 ± 1.4°C). *M. scutellaris* exited the nest with an LI (light intensity) varying between < 1 to 290 lux at nest door and from < 1 to 4,500 lux in the open air. Higher values occurred in the first activities of exiting the nest for *F. doederleini*. These bees exited the nest with an LI varying between 10 to 4,300 lux at nest door and from 290 to 19,200 lux in the open air.

In most of the observation months, the final times for entrance activity for *M. scutellaris* (17:00 – 18:00 h) coincided with sunset. These bees returned to the nest when the LI was low, around 1 lux. The final times for the daily flight activities of *F. doederleini* occurred approximately one hour earlier than for the other species (Fig 1), when the light intensity and temperature values were higher.

A daily activity rhythm for bee entrance and bee exit in the nest (without visible material) was detected for *M. scutellaris* in most of the study months, with highest activity (or acrophases) occurring during the morning, between 06:10 h (November 2009) and 08:35 h (July 2009), with the exception of March 2010, when the acrophases were observed during the afternoon, varying from 12:45 h (exit) and 13:35 h (entrance) (Table 2). During the observed days in March it was noticed a lot of rainfall. In relation to entrance activity with pollen, the acrophases for this species was in the morning, with times varying from 06:20 h (May 2010) to 08:50 h (July 2009) (Table 2).

*F. doederleini*’s acrophases for the activities of exit, entrance and entrance with pollen always occurred later than for *M. scutellaris* in all the study months and were mainly concentrated at the end of the morning (Table 2). For entrance and exit activities, the acrophases varied from 08:00 h (September 2009) to 11:35 h (March 2010). Preferential times for entrance activity with pollen occurred during the morning, varying from 08:50 h (January 2010) to 10:50 h (August 2009) (Table 2).

Fig 1 - Number of individuals of *Melipona scutellaris* and of *Frieseomelitta doederleini* entering with pollen in the nest throughout the day (between 400 and 1800 h) from July 2009 to March 2010 in the village of Pedra Branca (Santa Terezinha, Bahia).
Table 2: Acrophase times of exit and entrance activity (without visible material); entrance activity with pollen and entrance activity with resin for *Melipona scutellaris* and *Frieseomelitta doederleini* in the indicated months during 2009 and 2010, in the village of Pedra Branca (Santa Terezinha, Bahia).

<table>
<thead>
<tr>
<th>Bee/activity</th>
<th><em>Melipona scutellaris</em></th>
<th><em>Frieseomelitta doederleini</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exit</td>
<td>Entrance</td>
</tr>
<tr>
<td>Jul/09</td>
<td>07:00</td>
<td>08:35</td>
</tr>
<tr>
<td>Aug/09</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Sep/09</td>
<td>07:20</td>
<td>*</td>
</tr>
<tr>
<td>Nov/09</td>
<td>06:10</td>
<td>06:50</td>
</tr>
<tr>
<td>Jan/10</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Mar/10</td>
<td>12:45</td>
<td>13:35</td>
</tr>
<tr>
<td>May/10</td>
<td>07:05</td>
<td>07:25</td>
</tr>
</tbody>
</table>

*Values for non significant mean vector (r) (below 0.7).

During *M. scutellaris* acrophase times for the activities of exit, entrance and entrance with pollen, the temperature varied from 21 to 29°C (average 24 ± 2.0°C), the relative humidity from 56 to 99% (average 80 ± 9.8%) and the light intensity from 70 to 10,900 lux at nest door. For *F. doederleini*, the temperature varied from 24 to 29.3°C (average 27 ± 1.3°C), the relative humidity from 49 to 98% (average: 66 ± 12%) and the light intensity (nest door) from 180 to 6,900 lux.

*Melipona scutellaris* acrophases for entrance activity with resin varied from 05:10 h (November 2009) to 08:15 h (July 2009), while for *F. doederleini* this activity occurred between 11:25 h (August 2009) and 13:20 h (July 2009).

Discussion

Among the Meliponini colonies studied in Pedra Branca regular flight activity times were observed, both for their first activities and in the acrophases of most activities. This regularity may be related to several factors that are both extrinsic (abiotic) and intrinsic (biotic) to the bees or to an association between these factors. Several factors may determine the regularity of times for daily activities of bees. Some authors explain the regularity of time of these organisms through time-space learning, which attributes learning to the ability to return to sources of food at regular times. In stingless bees, Breed et al. (2002) state that *Trigona amalthea* (Olivier) is capable of associating location of food with its time of exposure. In addition, Biesmeijer et al. (1998) observed that *Melipona* spp.’s daily foraging activity is based on both previous experience, through which the bees are capable of retaining information about the external environment, and on social interactions within the colony.

This regularity, both in times for the beginning and end of activities and, mainly, in the flight acrophases of Meliponini species studied in Pedra Branca, may be related to the presence of a daily activity rhythm for the range of analysed activities, which occur at intervals of approximately 24 hours, at different times of the year. This daily activity rhythm may have an endogenous origin and may be a manifestation of the biological clock (Dunlap et al., 2004). The endogenous character of Meliponini species’ activity rhythm has been identified by maintaining colonies in constant laboratory conditions (Bellusci & Marques, 2001; Almeida, 2004).

Biological rhythms may be synchronized or entrained by environmental cycles, such as the light/dark (Dunlap et al., 2004). In Pedra Branca, the beginning and the end of *M. scutellaris* daily activities were associated with the hours of sunrise and sunset and generally occurred earlier in November, when the sun rises earlier, and later in July, when the sun rises later. Thus, the daily light/dark cycle may function to entrain the rhythm of daily flight activity in these bees. The entrainment of daily bee activities by the light/dark cycle was also observed by Bellusci and Marques (2001) in *Scaptotrigona aff depilis* foragers.

Few studies of the daily flight activity of bees in Brazil have a chronobiological focus. Hilário et al. (2003) detected the presence of daily rhythms in a range of activities in a *M. bicolor* Lepelletier colony in São Paulo, which occurred during the morning and differed according to the two seasons of the year - earlier in summer and later in winter. In a forest in Ubatuba (São Paulo), Fidalgo and Kleinert (2007) observed acrophases for collecting pollen in *M. rufiventris* Lepelletier (the majority being at the beginning of the morning) and suggested that this activity was related to time of sunrise. Rhythms for daily flight activity which repeat at intervals of approximately 24 hours may allow organisms to anticipate and “prepare” themselves for favourable or unfavourable environmental changes (Merrow et al., 2005) and are important in the ecological context, since bees may, for example, have foraging strategies, anticipating their visiting times in relation to the times of the opening of flowers and anthers (Gimenes et al., 1993; 1996).

Meteorological factors, such as temperature and relat-
tive humidity, may also modulate the bees’ external activities. The species of Meliponini studied did not leave the nest before the temperature reached 19°C, but for *M. scutellaris* acrophase times generally occurred when the average temperature was around 24°C and the acrophases of *F. doederleini* occurred at higher temperature values (average 27°C). Similar results have been obtained for *Frieseomelitta* flight activity in other localities, such as in Ribeirão Preto (São Paulo State) in a study of *F. doederleini* (under laboratory controlled conditions) (Almeida, 2004) and of *F. varia* (under field conditions) (Nevill et al., 2004).

Furthermore, positive correlation between bee activities of nest entrance with and without pollen and temperature may be observed in the studies of Souza et al. (2006), in relation to *M. asilvai* in Pedra Branca. However Fidalgo and Kleinert (2007) observed that in forest areas (São Paulo) the effect of temperature and relative humidity was different depending on the activity of the bee.

In this study the correlation between pollen collection and temperature was negative, but between nectar collection and temperature was positive. Nates-Parra and Rodrigues (2011) also observed negative correlation between entrance activity with pollen of colonies of *Melipona eburnea* Friese and temperature in the dry and rainy seasons and a negative correlation between relative humidity and entrance with pollen in the rainy season.

However, according to Silva and Ramalho (2011) the effect of relative humidity in the activities of stingless bees can be uncertain and casual, especially in humid tropical habitats. In this regard, we need more research about the effects of temperature and relative humidity in the flight activities stingless bees.

The differences observed between daily flight activity and temperature values related to the two bee genera may be related to their different size and colour, since the larger and darker bees presented earlier onset and acrophase times when the temperature values were lower, compared to the smaller sized and lighter bees (*F. doederleini*). Similar results were obtained by Bruijn and Sommeijer (1997) and Teixeira and Campos (2005), who worked with several Meliponini species of different sizes. When studying different Meliponini genera, Biesmeijer and Pereboom (2003) concluded that these bees’ thermal properties were significantly influenced by size and body colour, since the largest and darkest bees were able to regulate in lower temperature environments more easily than the smaller and lighter bees.

*M. scutellaris*’s first and last activities generally occurred close to sunrise and sunset, when light intensity values were low. *F. doederleini* carried out their first and last activities at higher levels of light intensity, since this bee began its activities approximately one hour after *M. scutellaris* and finished its activities earlier. The effect of light intensity on flight activities has already been observed in other species of Meliponini. When studying the *Trigona mosquito* (=*Plebeia mosquito*) (Smith), Lutz (1931) observed that light intensity interfered at the beginning and end of daily activities. Furthermore, in Ribeirão Preto (SP), Nevill et al. (2004) confirmed that this abiotic factor had a positive influence on the external activities of *F. varia*. According Corbet et al. (1993) the daily activities of social bees would start at sunrise and would be more correlated with light intensity than the temperature.

Compared to the other months, the excessive rain observed during the March observations did not result in a reduction in the amount of activity of the studied bees. Rain effect on flight activity of stingless bee could be different in other areas of Northeast Brazil. Nascimento and Nascimento (2012) observed in colonies of *Melipona asilvai* Moure, in a forest area (Sergipe), that daily activity was about 90% lower in the rainy season and they suggest that the colony enters into seasonal diapause at this time of the year. However, in March, was observed a change in the acrophase times for total bee’s activities, which occurred later than in the other months.

The acrophases for entrance activity with pollen, however, varied very little across the observation months. Maintenance of the times for entrance activity with pollen in March may be related to the possible regular time of exposure of this resource on anthers. According to Roubik (1989), in general, most plants present anther dehiscence at the beginning of the morning, which probably influenced the times the bees collected this resource. Imperatriz-Fonseca et al. (1985), working on the flight activity of *Plebeia remota* (Holmberg) in São Paulo, confirmed that the availability of floral resources is an external factor which may positively influence flight activity and could be a signal to the bees to regulate their activities.

Abiotic factors, such as light intensity, temperature and humidity, and biotic factors, such as time of food exposure, may influence the flight activity times of Meliponini species and may operate as modulating factors in the rhythm of activity. The direct response of the organism to an environmental stimulus allows it a flexibility that adds to the rigid entrainment mechanism, so that a modulating factor works in the fine adjustment of the synchronization for the biological rhythm (Marques & Waterhouse, 1994). According to Dunlap et al. (2004), in some organisms, temperature cycles function as entraining agents on the biological clock, but more often they perform a modulating role on the biological rhythm, over entrainment by the light/dark cycle.

The regularity of daily flight activities in Meliponini species studied, as well as the maintenance of acrophases in bees with differences in external morphologies, from colonies at the same stage of development, strengthens the concept that these bees have a biological rhythm.

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References


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