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Environmental windows for foraging activity in stingless bees, *Melipona subnitida* Ducke and *Melipona quadrifasciata* Lepeletier (Hymenoptera: Apidae: Meliponini)

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Abstract

The foraging success of a bee species is limited to an environmental window, a combination of optimal ambient temperatures and resource availability. Mismatches between flowering and optimal foraging temperature may lead to a reduction of a colony's food intake and, eventually, of brood production. In the present study, we evaluated the pollen foraging activity of two native Brazilian meliponine species *Melipona quadrifasciata* Lepeletier and *Melipona subnitida* Ducke at the campus of the University of São Paulo at Ribeirão Preto (March, 2010 – January, 2011). Whereas *M. quadrifasciata* naturally occurs in the study region (Brazilian Southeast), *M. subnitida* is restricted to the Brazilian Northeast. This difference in geographic distribution and concordant climatic specializations suggest differences concerning the environmental window between the two species. We investigated potential differences between the species concerning the thermal window within which foraging occurs, and associated differences in foraging activity, visited pollen sources, and colony survival. The lower temperature limit for *M. subnitida* (17 ° C) was 5 °C above the lower temperature limit found in *M. quadrifasciata* (12 °C). This difference resulted in a considerable time lag concerning the onset of foraging between the bee species (maximum: 120 minutes), mainly during the cold-dry season. Due to this delay in foraging, *M. subnitida* could not benefit from highly profitable pollen sources (mass-flowering trees) that were in bloom during this time of the year. Possibly because of this deficit in pollen intake, three of the six monitored colonies of *M. subnitida* did not survive the study period.

Introduction

Due to their impact on many aspects of colony life, abiotic factors are considered key determinants for the geographical distribution of social bee species (Michener, 1974). An environmentally influenced aspect of vital importance for colony functioning is food collection. The success of foragers, which is crucial for the maintenance and survival of the colonies, is affected both directly and indirectly by climatic factors. Associated with the morphological and interrelated physiological peculiarities of a bee species – such as body size and colouration – abiotic factors determine the timing of food collection (daily onset and end) and the food patch choice (sunny versus shaded patches) (Biesmeijer et al., 1999; Pereboom & Biesmeijer, 2003; Hrncir & Maia-Silva, 2013). In addition to this direct influence of climatic factors, primarily of ambient temperature, on the foraging activity of a species, climatic factors affect the flowering phenology of plants and, consequently, the availability of floral resources for the bees. Hence, the foraging success of a bee species is restricted to an environmental window (EW), a combination of optimal ambient temperatures and resource availability (Stone et al., 1999; Hilário et al., 2000). Mismatches between flowering and optimal foraging temperature may lead to a dramatic reduction of a colony's food intake and, eventually, of brood production, which depends on the availability of resources within the nest (Ribeiro et al., 2003; Ferreira-Junior et al., 2010).

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Given the necessity of an accurate match between resource availability and foraging temperature (Stone et al., 1999), the breadth of the environmental window of a bee species is determined by its capacity to acclimate to different abiotic conditions, the range of temperatures at which foragers may be active, and the dietary niche breadth. Due to the bigger amplitude of foraging possibilities, the foraging success of species with broad EWs, such as the honey bee *Apis mellifera* Linnaeus (Apidae, Apini), is bound to be less affected by variations in the abiotic and biotic environment than that of species with narrow EWs. This, on the one hand, should result in a wider geographic distribution of broad EW-species compared to narrow EW-species. On the other hand, geographic ranges should be more dynamic for narrow EW-species than for broad EW-species. Long-lasting climatic changes, such as global warming foreseen for the coming decades (Marengo et al., 2009; Nobre, 2011), may result in shifts in the geographic distributions particularly of narrow EW-species and, in consequence of the mutualistic interactions, of the plants they forage on (Guisan & Thuiller, 2005; Hegland et al., 2009).

An interesting group to study environmental windows are the stingless bees (Apidae, Meliponini), a group of highly eusocial bees with pantropical distribution (Michener, 1974; Michener, 2000; Camargo & Pedro, 2013). In contrast to *A. mellifera*, most meliponine species occur in rather narrow geographic ranges (Camargo & Pedro, 2013), which is typically attributed to physiological limitations and concomitant environmental specializations of the species. Given their ecological importance as pollinators of many native plant species (Imperatriz-Fonseca et al., 2012), it has become a major concern to understand the possible impact of climatic changes on these bees (Giannini et al., 2012). Here, knowledge of the environmental windows of key species may form a solid background for the development of successful conservation plans.

In the present study, we investigated the foraging activity of two meliponine species, *Melipona quadrifasciata anthidiodes* Lepeletier and *M. subnitida* Ducke, at the campus of the University of São Paulo at Ribeirão Preto-SP. Of these, *M. quadrifasciata* naturally occurs in the study region in the Brazilian Southeast (Camargo & Pedro, 2013), whereas the occurrence of *M. subnitida* is restricted to the Brazilian tropical dry forest, the Caatinga, in the Brazilian Northeast (Zanella, 2000). This difference in geographic distribution and concordant climatic specializations suggest that differences in the environmental window of these two species may exist. Monitoring the pollen foraging activity of *M. quadrifasciata* and *M. subnitida* during 11 months, we tried to answer the following questions: (1) Is there a difference between the species concerning the thermal window within which foraging occurs and, if so, does this difference result in differences in foraging activity? (2) Is there a difference concerning the pollen resources collected by the species and, if so, may this be attributed to differences in foraging activity? (3) Is there a difference concerning colony survival between *M. quadrifasciata* and *M. subnitida* and, if so, may this be explained by differences in resource access?

Material and Methods

Study site and period

The study was conducted from March, 2010 through January, 2011 at the experimental meliponary of the campus of the University of São Paulo at Ribeirão Preto-SP (21°10’30” S, 47°48’38” W) in the Brazilian Southeast. The vegetation of the university campus is composed of both native species of seasonal semi-deciduous forest and exotic plants (vegetation cover of the campus: ~ 75 ha; Pais & Varanda, 2010). The area directly surrounding the meliponary presents a high diversity of potential food sources for bees (Faria et al., 2012; Aleixo et al., 2013). The local climate is characterized through two well-defined seasons, a hot-rainy season from September/October to April, and a cold-dry season from May to August/September. Throughout our study, ambient temperature, relative humidity and precipitation were monitored by a weather station (WMR982, Oregon Scientific Inc., U.S.A.) installed near the meliponary.

Bee species

For our study, we monitored six colonies of *M. quadrifasciata* (MQ) and six colonies of *M. subnitida* (MS). All bee colonies were housed in wooden observation hives that had been installed at the meliponary at least 3 months prior to the onset of the study. MQ naturally occurs in the study area, characterized through seasonal semi-deciduous forest vegetation and, climatically, through two well-defined seasons (cold-dry season, hot-rainy season) (Camargo & Pedro, 2013; Oliveira et al., 2013; Aleixo et al., 2014). Workers are 8 to 10.5 mm in length and have a thorax with of between 3.75 and 4.75 mm (Schwarz, 1932). MS naturally occurs in the Caatinga in the Brazilian Northeast, climatically classified as semi-arid with elevated annual temperatures and extended periods of drought (Prado, 2003). This exclusively Brazilian biome is characterized through tropical dry forest and scrub vegetation (Sánches-Azofeita et al. 2013). Workers of this species are 7.5 to 8.5 mm in length and have a thorax with of 3.75 mm (Schwarz, 1932).

Pollen foraging activity

We evaluated the foraging activity of two of the monitored MQ colonies and of at least three colonies of MS by counting the number of foragers returning to the nests with pollen loads between 5:30 am and 5:30 pm. During peak activity (usually, between 5:45 am and 10:00 am), the number of pollen foragers was registered for 5 minutes every 15 minutes. When the colonies' foraging activity decreased, bee counts were made for 5 minutes every 30 minutes (usually, between 10:00 am and 12:00 pm) and for 5 minutes every 1 hour (usually, between 12:00 pm and 5:30 pm). Depending on the general activity of the colonies, bee counts were made on between 4 and 7 days per colony in each month.

Floral origin of pollen collected by bees

In order to evaluate the floral origin of pollen collected by the bees, foragers with pollen loads were captured on their return to the nests. Pollen sampling was performed twice or, when activity was high, three times between 6:00 am and 9:00 am on days on which we did not investigate the foraging activity. For the sampling, we blocked the nest entrances for a maximum of five minutes, and captured the returning pollen foragers individually in plastics vials when they tried to enter the closed colony. In order to avoid a significant reduction of the pollen foraging force on subsequent days, we caught a maximum of three bees during each collecting event (maximum of 9 bees per colony per day). The captured bees were chilled on ice for 5 minutes to reduce their mobility and facilitate manipulation. Subsequently, the pollen pellets were removed from the bees' corbiculae with alcohol-cleaned tweezers and stored in test tubes. Thereafter, the foragers were released. After acetolysis of the individual pollen samples (method described by Erdtman, 1960), the floral origin of the pollen loads was identified through comparison with reference material from university's pollen collection. To evaluate the relative composition of potentially mixed pollen loads, we identified the floral origin of 400 pollen grains of the pellets of each forager (Nagamitsu et al., 1999). Samples containing between 95% and 100% of pollen grains of the same floral source were considered as pure samples (Eltz et al., 2001). Most of the samples consisted of only one type pollen (pure samples). In samples containing two or more pollen types, we regarded the most abundant type as the respective forager's principal pollen source (Nagamitsu et al., 1999).

Analysis of the thermal window of pollen foraging

To assess the preferred temperature range for pollen collection, we evaluated the number of foragers returning to their nest at a given ambient temperature (to the nearest °C). This method slightly overestimated the actual foraging temperatures of the individuals because ambient temperatures steadily increased in the course of our observations and, thus, incoming pollen collectors, which forage for several minutes, were registered at the maximum temperature of their foraging trip. Nonlinear Regression Analysis (Gaussian Peak Model) with ambient temperature as predictor and the percentage of returning foragers as dependent variable was used to evaluate the preferred temperature range for pollen collection. From the regression model, we obtained the thermal window of both investigated species (temperature range within which 90% of activity occurs). The potential difference between the thermal windows of MQ and MS was evaluated using a Mann-Whitney Rank Sum Test.

Analysis of pollen foraging activity

To evaluate the foraging activity of the investigated bee species, we calculated for each month the following parameters of foraging activity: F_{ON}, average time of foraging onset, F_{END}, average end of pollen foraging activity, and F_{MAX}, average maximum number of foraging bees. $F_{ON,}$ was the first 5 minute-interval in which we registered incoming pollen foragers. F_{END} was the last 5 minute-interval in which we registered returning pollen foragers, followed by at least two 5 minute-intervals with zero-counts. For the statistical analyses, time data (hour:minute) were transformed into decimal numbers (hour + minutes/60, so that, e.g., 05:50 am became 5.83). Potential differences in foraging activity parameters between MQ and MS were evaluated using Paired *t*-tests (monthly average value for MQ paired with monthly average value for MS at a given month). Monthly differences between the two bee species concerning the timing of foraging activity were described through the time lag between the foraging onsets (time lag = $F_{ON-MS} - F_{ON-MQ}$).

Analysis of pollen resource diversity and overlap

For each month of our study, we assessed the plants visited by MQ and MS to collect pollen. From these data, we calculated the monthly resource diversity of MQ and MS through Shannon's Diversity Index, H'. Potential differences concerning resource diversity between the bee species were evaluated using a Paired *t*-test (monthly H' for MQ paired with monthly H' for MS at a given month). Additionally, we evaluated the monthly overlap in collected pollen sources between MQ and MS using the Morisita-Horn Overlap Index, C_H ($C_H = 0$, no overlap; $C_H = 1$, complete overlap). To determine whether and to which degree differences in resource use are associated with differences in foraging activity between the species, we evaluated the correlation between resource overlap (C_H) and time lag of foraging onset using Pearson Product Moment Correlation.

Analysis of colony survival

Monthly, we evaluated the status of the monitored colonies (6 colonies MQ, 6 colonies MS). A colony was considered as "dead", in case no queen or worker bees were found in the colony.

Statistical analysis

All statistical analyses were performed using the software packages SigmaPlot 10.0/SigmaStat 3.5 (Systat Software Inc., U.S.A.) and Statistica 8.0 (StatSoft Inc., U.S.A.). The α-level for significant differences was $P \leq 0.05$.

Results

Climatic variations

In the course of our study (March, 2010 through January, 2011), average temperatures varied by about 8 °C (16.8 °C – 24.5 °C). In the cold-dry season (May, 2010 through August, 2010), minimum temperatures were between 5.2 to 7.1 °C and maximum temperatures between 31.1 and 37.4°C. The total precipitation in this period was 38.0 mm. In the hot-rainy season (Mach, 2010 through April, 2010, and September, 2010 through January, 2011), maximum temperatures (32.9 – 38.0 °C) did not differ much from maximum temperatures in the cold-dry season. Minimum temperatures, by contrast, were considerably higher in the hot-rainy season (10.5 – 19.6 °C) than in the cold-dry season. The total precipitation during this period was 660.2 mm. During our study, average relative humidity remained above 60% (60.2 – 78.0 %) with exception of August (46.7%) and September (55.0%). In Table 1, the monthly values of climatic variables obtained in the course of our study are presented in detail.

Table 1. Variation of environmental variables (EV) in the course of our study. Given are the respective values for each month: T_{AVG}, average temperature; T_{MAX}, maximum temperature; T_{MIN}, minimum temperature; RH_{AVG}, average relative humidity; RAIN, total precipitation.

	Month										
EV	03/10	04/10	05/10	06/10	07/10	08/10	09/10	10/10	11/10	12/10	01/11
T_{AVG} (°C)	24.0	21.2	18.1	16.8	19.1	20.2	23.2	22.2	23.2	24.5	24.0
T_{MAX} (°C)	33.6	32.9	31.4	31.1	31.9	37.4	38	37.7	35.4	35.2	35.6
T_{MIN} (°C)	16.5	11.3	5.3	5.2	7.6	7.1	10.5	10.5	12.3	17.2	19.6
RH_{AVG} (%)	67.2	73.3	72.7	67.9	60.2	46.7	55	60.2	67.2	76.2	78
RAIN (mm)	128.3	48.1	14.2	7.9	15.9	0.0	98.2	58.2	127	109.1	91.3

Thermal window of pollen foraging

Analysing the number of foragers returning to the colonies at a given ambient temperature, we observed a difference between *M. quadrifasciata* (MQ) and *M. subnitida* (MS) concerning the temperature range at which foraging occurs (Fig. 1). The thermal window for pollen foraging (90 % of returning foragers) of MQ (*N* = 2 colonies; *n* = 3,295 foragers) was between 12 and 22 °C (maximum foraging activity calculated by Gaussian Peak Model-Analysis: 17.7 °C), and that of MS (*N* = 6; *n* = 2,772) was between 17 and 24 °C (maximum foraging activity: 19.3 °C). This difference was statistically significant (Mann-Whitney Rank Sum Test: $U = 2{,}312{,}623.5$; $P < 0.001$).

Pollen foraging activity

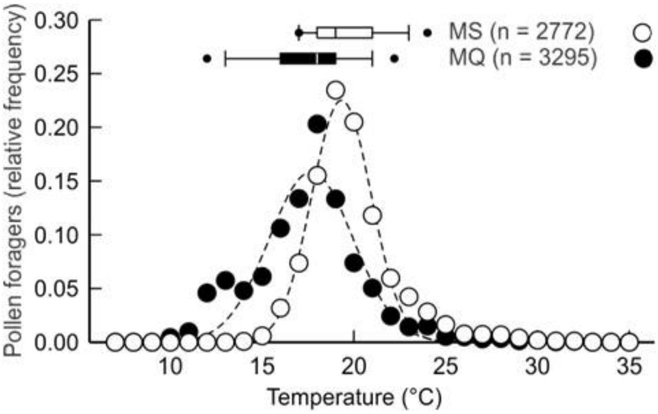

Fig 1. Thermal window of pollen foraging. Scatterplot shows the amount of pollen foragers (proportional frequency relative to the total number of evaluated foragers) of *Melipona quadrifasciata* (MQ, filled circles and box) and *M. subnitida* (MS, open circles and box) returning to the colonies at a given ambient temperature (to the nearest ºC). Dashed lines indicate the respective Gaussian Peak-Models. Horizontal Boxplot indicates the median temperature (line within box), and the temperature ranges in which 50 % (box), 80 % (whiskers), or 90 % of the foragers (outliers) returned to to the colonies with pollen loads.

During our study, the pollen foraging activity of the investigated colonies of MQ initiated between 5:30 am (December, 2010) and 7:15 am (July, 2010) and ended between 6:15 am (December, 2010) and 10:30 am (July, 2010). The colonies of *M. subnitida* started (6:00 am – 8:45 am) and ended (8:30 am – 11:15 am) their pollen collection significantly later than MS (Paired *t*-test: F_{ON}, $t = -3.94$, $df = 8$, $P = 0.004$; F_{END}, $t = -4.73$, $df = 8$, $P = 0.001$). The time lag between the foraging onsets of MQ and MS was between 4 minutes (November, 2010) and 2 hours (August, 2010) (Fig 2A).

In two months of our study (May and June, 2010), both bee species showed almost no pollen collection activity (Fig 2B). Consequently, we could not evaluate the timing of foraging for these months. In most of the other months, the maximum number of pollen foragers (F_{MAX}) of MQ was higher than that of MS. Despite a significantly higher F_{MAX} of MQ compared to MS considering the entire study period (Paired *t*-test: $t = 2.44$, $df = 10$, $P = 0.035$), the maximum foraging force of both species was very similar in September and October, and in December, F_{MAX} of MS was even higher than that of MQ (Fig 2B).

Diversity and overlap of pollen resources

We were not able to collect pollen from foragers in May and June, 2010 (virtually no pollen foragers) and in November, 2010 and January, 2011 due to heavy rainfall on collection days that impaired pollen foraging. In the course of the evaluable months, MQ collected pollen at 23 plant species (between 3 and 11 species in each month) and MS at 18 plant species (between 3 and 8 species in each month) (Fig 3A; Table 2). The average diversity of collected resources did not differ significantly between MQ and MS ($H'_{MQ} = 1.39 \pm 0.45$; $H'_{MS} = 1.40 \pm 0.36$; Paired *t*-test: $t = -0.05$, $df = 6$, $P = 0.962$).

In March, September, and October, MQ and MS collected pollen virtually at the same plant species (almost complete

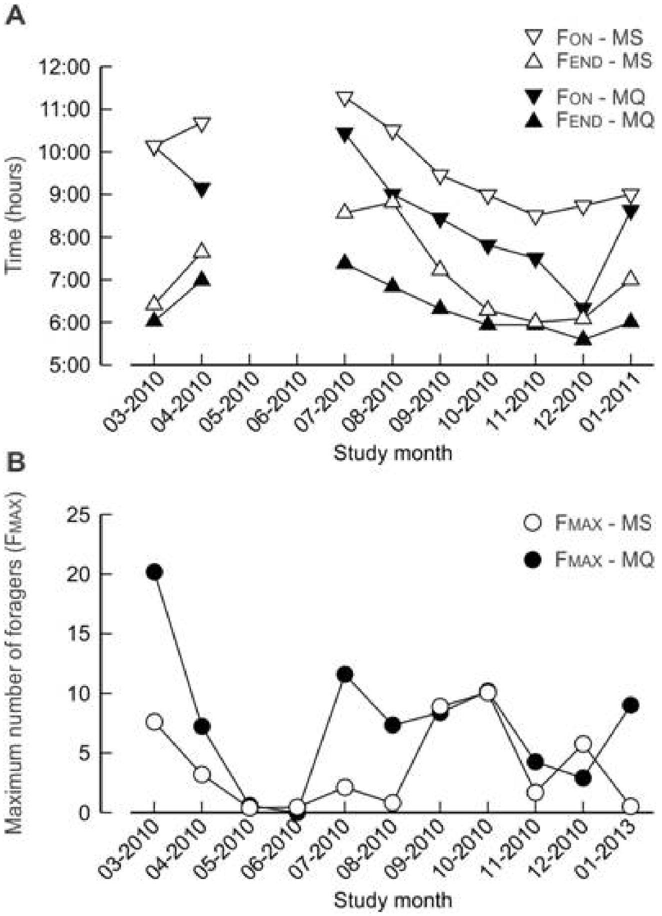

Fig 2. Pollen foraging activity of *Melipona quadrifasciata* (MQ, filled symbols) and *M. subnitida* (MS, open symbols) in the course of our study. (**A**) Monthly average onset (F_{ON}) and end (F_{END}) of pollen foraging. (**B**) Monthly average maximum of pollen foragers (F_{MAX}). N_{MQ} = 2 colonies; N_{MS} = 4 colonies (03-2010 to 06-2010) and 3 colonies (07-2010 to 01-2011).

resource overlap; C_H = 0.91 – 0.94). On the other hand, resource overlap was low in July (C_H = 0.28), August (C_H = 0.00), and December (C_H = 0.40). The average resource overlap was 65 % (C_H = 0.65 ± 0.39) (Table 2). Resource overlap decreased with increasing time lag between the foraging activity of MQ and MS (Pearson Product Moment Correlation: R = -0.88, P = 0.010, n = 7 months evaluated) (Fig 3B).

Colony survival

All six monitored colonies of MQ survived until the end of our observations. By contrast, three of the six monitored colonies of MS died in the course of our study (one colony in July, 2 colonies in August).

Discussion

In the present study, we evaluated the pollen foraging activity of *M. quadrifasciata* and *M. subnitida* at the campus of the University of São Paulo at Ribeirão Preto. Whereas *M. quadrifasciata* naturally occurs in the study region in the Brazilian Southeast, the geographic distribution of *M. subnitida* is restricted to the Brazilian tropical dry-forest in the Northeast of the country (Zanella, 2000; Camargo & Pedro, 2013), which is characterized through elevated annual temperatures and an extended hot-dry season. This limited occurrence of *M. subnitida* suggests strong environmental specialisations and survival strategies to cope with the long and often irregular periods of drought (Maia-Silva, 2013). In spite of the fact that this meliponine species has been repeatedly introduced into different environments (including São Paulo state) for beekeeping or research purposes (Nogueira-Neto, 1997; Koedam et al., 1999), these attempts often resulted in the loss of all established colonies (Nogueira-Neto, 1997). The results of our study now indicate that the possible reason for these and similar failures is the mismatch between optimal ambient temperature for foraging and resource availability mainly in the cold-dry season.

The temperature range of *M. subnitida* within which pollen foraging occurred was between 17 and 24 °C. In its natural habitat, the Brazilian tropical dry forest, the thermal window for pollen foraging of this bee was found to be between 21 and 29 °C (Maia-Silva, 2013). Yet, despite the apparent capacity of acclimatization to the lower ambient temperatures in the Brazilian Southeast (minimum temperatures 5 to 20 °C) as compared to the Brazilian Northeast (minimum temperatures 18 to 21 °C; Maia-Silva, 2013), the low-temperature threshold for *M. subnitida* was still 5 °C above the low-temperature threshold of *M. quadrifasciata* (12 °C). This difference concerning the thermal window between the two meliponine species is presumably related to the bees' physiological adaptations to the climatic situation of their respective natural habitats. Here, a critical factor is the absolute physiological limit of bees, determined by the temperature below which endothermic heating of the flight muscles becomes uneconomic (Heinrich, 1993; Stone, 1993; Stone et al., 1999).

Table 2. Resource diversity and overlap. Given are the numbers of pollen types collected by *Melipona quadrifasciata* (MQ) and *M. subnitida* (MS) in each month of our study (n indicates the number of analyzed foragers). From these, we calculated the resource diversity (Shannon Diversity Index, H') for each bee species and the resource overlap (Morisita-Horn Overlap Index, CH) between species.

	Number of collected pollen types		Shannon Diversity Index (H')		Morisita-Horn Overlap Index (C_H)
Month	MQ (n)	MS (n)	MQ	MS	MQ – MS
03-2010	07 (18)	08 (32)	1.72	1.80	0.91
04-2010	07 (19)	06 (41)	1.65	1.38	0.51
05-2010	- (0)	- (0)	-	-	-
06-2010	- (0)	- (0)	-	-	-
07-2010	11 (24)	08 (11)	2.09	1.97	0.28
08-2010	03 (20)	04 (06)	0.82	1.33	0.00
09-2010	05 (19)	04 (12)	1.16	1.20	0.92
10-2010	04 (25)	04 (24)	1.05	1.01	0.94
11-2010	- (0)	- (0)	-	-	
12-2010	04 (13)	03 (18)	1.23	1.07	0.98
01-2011	- (0)	- (0)	-	-	
Average ± SD	-	-	1.39 ± 0.45	1.40 ± 0.36	0.65 ± 0.39
Total	23 (138)	18 (144)	2.68	2.28	0.74

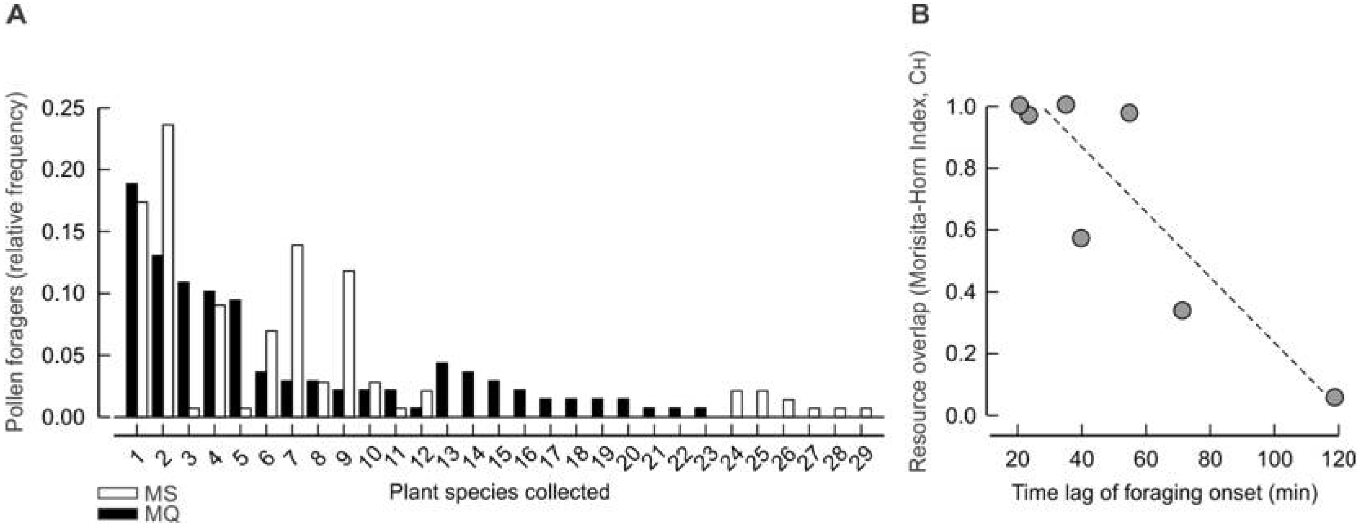

Fig 3. Pollen source use and overlap. (**A**) Bar-chart shows the amount of foragers (proportional frequency relative to the total number of evaluated foragers) of *Melipona quadrifasciata* (MQ, filled bars, *n* total = 138 analyzed pollen foragers) and *M. subnitida* (MS, open bars, *n* total = 144) returning to the colonies with pollen collected at the following plants: 1 - *Anadenanthera peregrina*; 2 - *Mimosa* sp.; 3 - *Eugenia pyriformis*; 4 - *Eugenia involucrata*; 5 - *Eugenia uniflora*; 6 - *Eucalyptus moluccana*; 7 - *Leucaena leucocephala* ; 8 - *Eugenia brasiliensis*; 9 - *Eucalyptus grandis*; 10 - *Solanum* sp.; 11 - *Cecropia pachystachya*; 12 - *Pterocarpus violaceus*; 13 - *Solanum cernuum*; 14 - *Solanum paniculatum*; 15 - *Syzygium malaccense*; 16 - *Albizia lebbeck*; 17 - *Capsicum baccatum*; 18 - *Eucalyptus* sp.; 19 - *Solanum seaforthianum*; 20 - *Vernonia* sp.; 21 - Indeterminate 1; 22 - *Lagerstroemia indica*; 23 - *Serjania lethalis*; 24 - *Citrus limonia*; 25 - *Senegalia polyphylla*; 26 - *Eucalyptus citriodora*; 27 - *Handroantus* sp.; 28 - Indeterminate 2; 29 - *Psidium guajava*. (**B**) Scatterplot showing the correlation between the monthly average time lag concerning the onset of pollen foraging between *M. subnitida* and *M. quadrifasciata* and the monthly resource overlap (Morisita-Horn Index, C_H) between the two investigated bee species. See results for details on the correlation analysis.

The observed differences concerning the low-temperature threshold between *M. subnitida* and *M. quadrifasciata*, and the consequent differences in foraging onset (Fig 2), led to a segregation of the utilized resources among the species particularly in the coldest months of our study (Fig 3). Whereas both bee species forged at virtually the same plants in months with elevated ambient temperatures (high resource overlap in March, September, October, December), *M. subnitida* missed out on important pollen sources visited early in the morning by *M. quadrifasciata* in months with low morning temperatures. Owing to a low-temperature threshold of 12 °C, *M. quadrifasciata* was able to initiate foraging before 7:00 am in July and August (minimum temperatures < 8 °C at around 5:00 am). In these months, *M. quadrifasciata* foragers collected pollen predominantly at the mass-flowering trees *Eugenia pyriformis* and *E. uniflora* (July: *E. pyriformis*: 33.3% of the evaluated pollen; August: *E. pyriformis*: 35% of the evaluated pollen; *E. uniflora*: 60% of the evaluated pollen). Mass-flowering plants, in general, produce an excessive number of flowers each day, thus providing large amounts of pollen and/or nectar to flower visitors (Gentry, 1974; Bawa, 1983). For stingless bees, mass-flowering plants offer an excellent opportunity to amass floral resources within their nests and are the predominant source of nectar and pollen, contributing by up to 90% to the annual nutritional input into the colonies (Wilms et al., 1996; Ramalho, 2004).

The flowers of *E. pyriformis* and *E. uniflora* open early in the morning, around 5:00 am (Proença & Gibbs, 1994), and are rapidly exploited by bees before 8:00 am to 9:00 am (Silva & Pinheiro, 2007). In contrast to *M. quadrifasciata*, *M. subnitida* initiated foraging in July and August not before 8:30 am. Thus, due to their elevated low-temperature threshold, the colonies of *M. subnitida* started pollen collection close to the time the mass-flowering pollen bonanzas became unprofitable. This observed mismatch between the thermal window of *M. subnitida* and the availability of mass-flowering food sources was presumably the main cause for the loss of 50% of the colonies. Owing to extremely low ambient temperatures in the morning, both studied bee species did not collect pollen in May and June. Consequently, the access to mass-flowering trees in the subsequent months was crucial for the bees to refill their pollen storage and resume their brood cell-construction, and, eventually, decisive for colony survival.

The importance of highly profitable pollen plants, such as mass-flowering trees, for both *M. quadrifasciata* and *M. subnitida* became evident when evaluating the pollen collected by the colonies. The vast majority of the foragers' pollen loads originated from mass-flowering plants belonging to the botanical families Myrtaceae and Fabaceae (*M. quadrifasciata*: 80% of pollen loads, 12 plant species; *M. subnitida*: 93% of pollen loads, 13 plant species). Additionally, the bees collected pollen at plants with poricidal anthers belonging to the family Solanaceae, which are highly attractive for bees owing to the high quantity of pollen present in the flowers (Buchmann, 1983) (*M. quadrifasciata*: 12% of pollen loads, 4 species; *M. subnitida*: 2% of pollen loads, 1 species). Thus, despite the availability of close to 300 plant species belonging to 73 botanical families at the university's campus (Aleixo et al., 2014), the bees showed a high selectivity and floral preference for plants providing high amounts of pollen.

The apparent dependency and specialisation of *Melipona* bees on highly profitable pollen sources underlines the importance of an accurate match between the bees' thermal window and the availability of these resources. Here, species with broad environmental windows (broad EW-species) have considerable advantages over species with narrow thermal windows (narrow EW-species). Owing to a wider temperature range within which foraging may occur, broad EW-species are less affected by thermal variations in the environment than are narrow EW-species (Stone et al., 1999; Hilário et al., 2000). This, on the one hand, results in a wider geographic distribution of broad EW-species, such as *M. quadrifasciata* (thermal foraging window: 12 – 22 °C; amplitude: 10 °), which occur from the Brazilian Southeast to Northeast, compared to narrow EW-species, such as *M. subnitida* (thermal foraging window: 17 – 24 °C; amplitude 7 °), which are restricted to parts of the Brazilian Northeast (Zanella, 2000; Camargo & Pedro, 2013). On the other hand, broad EW-species may be less affected by long-lasting climatic changes, such as global warming predicted for the coming decades (Marengo et al., 2009; Nobre, 2011) than are narrow EW-species. Increasing ambient temperatures presumably lead to shifts in the timing of both flowering and pollinator activity (Memmott et al., 2007; Hegland et al., 2009). These phenological responses to climate warming may occur at similar magnitudes in plants and bees, thereby maintaining existent mutualistic plant-pollinator relationships. However, broad EW-species presumably accompany phenological shifts of plants better than narrow EW-species that may suffer from any tiny temporal mismatch between the timing of flowering and foraging activity (Memmott et al., 2007; Hegland et al., 2009), as was the case in our study. So far, mismatches in pollination interactions have been poorly studied (Hegland et al., 2009). Here, the determination of the environmental window of a bee species may serve as important basis for understanding the potential decoupling of pollinator activity from the timing of flowering and its consequences for ecosystem functioning or species distribution.

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