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Partitioning of pollen resources by two stingless bee species in the north Bahia, Brazil

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Abstract

Analysing the pollen stored by stingless bees allows identification of the diversity of flowering plants visited by these bees while collecting resources during a given period. However, few studies have focused on investigating the sharing of resources between species of native bees that coexist in transitional plant formations, which support conservation efforts and increase colony productivity. This study aims to describe the partitioning of pollen resources among colonies of two species of stingless bees in the state of the Bahia (Brazil) through the analysis of stored pollen. The study was conducted in neighbouring vegetation zones of the relevant species *Melipona quadrifasciata anthidioides* and *Melipona scutellaris* by collecting monthly pollen samples stored by three colonies of each species over one year. The collected samples were treated using the acetolysis technique, and the partitioning of pollen resources between the two species was investigated through qualitative analysis of the treated pollen. The results show that among the 16 pollen types belonging to a particular species or group identified in the *Melipona quadrifasciata anthidioides* samples and the 18 identified in the *Melipona scutellaris* samples, 13 were shared by both species. The two most common pollen types stored by both species were from *Mimosa arenosa* (Fabaceae, Mimosoidae) and *Eucalyptus* sp. (Myrtaceae). These results demonstrate the niche similarity of pollen pasture between these two species and suggest that conservation efforts for these bee species should focus on the plant families most visited by bees.

Keywords: *Melipona quadrifasciata anthidioides*, *Melipona scutellaris*, *pollen*

The keeping of social stingless bees (beekeeping) is a low environmental impact activity that produces highly nutritional food and a guaranteed financial return when the correct handling techniques are observed. The positive impacts of beekeeping include environmental services such as pollination, contributions to the maintenance of plant and animal communities and the possibility for economic exploitation of bee products (Velthuis 1997).

The reduction in habitats has significantly affected bee survival. Moreover, disturbed areas have been increasingly used for beekeeping with the goal of producing honey and pollen. These activities require

investigating bee foraging behaviour to learn which plant species the bees visit and what resources they collect. Such information is relevant to help producers make decisions, and to ensure that species are planted that promote both bee conservation and increased production.

Some studies in forest formations have shown the importance of stingless bees (Meliponini) as pollinators in native flora. However, stingless bees remain understudied compared to better-known relatives such as bumblebees and honeybees. This lack of comprehensive study is in part due to the diversity of stingless bees – there are more than 500 known

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stingless bee species in the tropical regions (Aguiar et al. 2014).

Understanding the floral source of pollen stored by social stingless bees assists in recognising the melliferous and pollen-producing plants that stingless bees use for trophic resources. The qualitative analysis of pollen enables the identification of the plant families most used by bees (Iwama & Melhem 1979; Barth 1989) and is particularly important in contributing to knowledge of the local flora and developing preservation techniques for the plant species native bees visit most – especially considering the worldwide decline of the honeybee. Although this decline is due to complex factors, low-quality forage is among them (Aguiar et al. 2014).

Regarding the Meliponini tribe, the analysis of palynological results in Brazil points to a variety of trophic resources and generalised habits of pollen harvesting (Barth 2004). In contrast, regional studies show that Meliponini frequently collect pollen from only a few floral sources (Ramalho et al. 1990).

Among stingless social bee species that occur in Brazil, those belonging to the genus *Melipona* must be noted, including the species *M. scutellaris* Latreille and *M. quadrifasciata anthidioides* Lepeletier, which originate from the regions where they occur naturally in Bahia State (Alves et al. 2006). *Melipona scutellaris* lives in humid and seasonal forests and is well known for its honey-producing prowess. *Melipona quadrifasciata anthidioides* inhabits the Caatinga biome and the seasonal forest and is considered the most productive species of Meliponinae in the dry areas of the Brazilian northeast.

Food resource availability plays an important role in limiting where different meliponines can live, grow, reproduce and coexist (Oliveira-Abreu et al. 2014). Different species often share the same floral sources, although not necessarily during the same foraging period (Novais et al. 2015). This study aims to investigate how resources are partitioned between two bee species in an anthropised seasonal forest region to gain knowledge that can help efforts to subsidise the husbandry of native bee species and influence future conservation policies.

Material and methods

The experiment was developed at Universidade Federal do Recôncavo da Bahia (UFRB), in the experimental area of the Núcleo de Estudos dos Insetos (INSECTA) of the Centro de Ciências Agrárias, Ambientais e Biológicas (CCAAB), at the campus of Cruz das Almas, Bahia, Brazil. The study area is within the distribution range of both *Melipona quadrifasciata anthidioides* and *M. scutellaris*, in transitional vegetation of a seasonal forest (Caatinga). The area

is strongly anthropic, with a semi-humid climate. It is classified as type *Af* on the Köppen Climate Classification, displaying two distinct seasons: a drought (August–February, characterised by high temperatures and sporadic but intense rainfall) and a rainy season (March–July, with lower temperatures and constant rain) (Agritempo 2015).

The studied colonies of *Melipona quadrifasciata anthidioides* and *M. scutellaris* were installed in rational INPA-type boxes containing nest, over nest and frames, previously demarcated, and one metre distant from each other. Stored pollen samples from three colonies each of *M. quadrifasciata anthidioides* and *M. scutellaris* were collected monthly during a 12-month period (March 2012–February 2013). The samples were collected from each colony by inserting a perforated tube (auger) vertically into the bottles of pollen stored by bees and removing a section of content. The collected pollen sample in the tube was extracted with the aid of a spatula and transferred to individual Falcon tubes. To document the collection of pot samples, a photographic record was made at the time of collection using a Canon HS100 camera. With each new harvest, the pictures recorded from the previous collections were used to compare and identify newly built pollen-containing pots, to avoid duplicate collections from the same pot. During the experiment, there was no artificial pollen-based colony feeding.

The pollen was processed using the acetolysis method described by Erdtman (1952). The polleniferous sediment resulting from the acetolysis procedure was used to assemble microscope slides covered with glycerine jelly, which were then used in a qualitative analysis of the pollen grains. Then, the slides were micro-photographed. Qualitative analysis (pollen present in the samples) was determined by comparing the observed pollen with previously identified pollen in slides available in the collection of permanent pollen grain slides maintained by the Pollen Laboratory of the INSECTA Group (UFRB) and with descriptions obtained from palynological literature including Barth (1989, 2004, 2006) and Barth et al. (2005, 2006). To calculate the relative frequency and abundance classes, a technique from Louveaux et al. (1978) was used to estimate the average amounts of pollen found in the colonies of *Melipona quadrifasciata anthidioides* ($n = 3$) and *M. scutellaris* ($n = 3$).

The analysis of pollen grains of particular pollen types was performed by counting the number of pollen grains contained in each microscope slide to enable a comparison between the two species. Samples that showed the number of pollen grains smaller or greater than 1000 were made for the calculations necessary to determine the percentages of pollen types and their frequency (FR) according

Table I. Relative frequency (%) and abundance class of pollen types found in pollen samples stored by *Melipona scutellaris*.

Pollen types	2012												2013												Classes of abundance		
	Months																										
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Relative frequency (%)														
Euphorbiaceae	0.42	0.10											0.04												OIP		
<i>Croton heliotropifolius</i> Kunth.																											
Fabaceae (Mimosoideae)	1.92		0.78	5.48	1.12		0.05			3.32	0.15	0.04	1.07												OIP		
<i>Acacia bahiensis</i> Benth.	0.70		38.54							7.53			3.90												IIP		
<i>Acacia</i> sp.	17.45	7.00	1.56	26.67	8.58	55.19		17.32	25.11	45.57	52.23	16.81	22.79												AP		
<i>Mimosa arenosa</i> Poir.	0.42	33.40	4.17	0.17			44.81	0.91					6.99												IIP		
<i>Mimosa caesalpinjifolia</i> Benth.	0.42	1.87						2.83	14.47				1.63												OIP		
<i>Mimosa pudica</i> L.	28.15												2.35												OIP		
<i>Mimosa</i> 1													2.82												OIP		
<i>Mimosa</i> 2				33.82																							
Myrtaceae	49.66	49.07	43.49	33.41	6.84	35.24	2.92		60.42	0.04	3.65		23.73												AP		
<i>Eucalyptus</i> sp.													8.96												IIP		
<i>Psidium</i> sp.					38.44	7.44	1.07	28.5			13.12	19.01	6.21												IIP		
Myrtaceae type 1					44.26						30.25																
Sapindaceae																											
<i>Serjania pernambucensis</i> Radlk.						1.92	51.12	18.32		43.54			9.58												IIP		
Sapindaceae type 1								31.89				64.04	7.99												IIP		
Solanaceae																											
<i>Solanum</i> sp.		0.59		0.46	0.09								0.09												OIP		
Solanaceae type 1								0.04					0.01												OIP		
Unidentified types																											
NI1	0.42	7.39	10.42			0.15	0.03	0.15				0.04	1.55												OIP		
NI2	0.42	0.58	1.04		0.67	0.06		0.04			0.49	0.04	0.28												OIP		
NI3											0.10	0.04	0.01												OIP		
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100		

Note: AP, accessory pollen (15% < FR < 45%); IIP, important isolated pollen (3% < FR < 15%); OIP, occasional isolated pollen (FR < 3%); NI, not identified.

Table II. Relative frequency (%) and class of abundance of pollen types found in pollen samples stored by *Melipona quadrifasciata anthidioides*.

Pollen types	2012												2013		Classes of abundance
	Months												Relative frequency (%)		
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb			
Fabaceae (Mimosoideae)															
<i>Acacia bahiensis</i> Benth.	43.41		31.15				0.04	8.85		46.96	71.00		0.89	OIP	
<i>Mimosa arenosa</i> Poir.		61.79					50.14			3.04	29.00		24.27	AP	
<i>Mimosa caesalpinifolia</i> Benth.													9.38	IIP	
<i>Mimosa pudica</i> L.		13.33					48.31	76.72					13.84	IIP	
<i>Mimosa</i> sp.	6.85					49.76							5.66	IIP	
<i>Acacia</i> sp.			0.93				0.14	14.43					1.55	OIP	
Myrtaceae															
<i>Eugenia uniflora</i> L.			67.29										6.73	IIP	
<i>Eucalyptus</i> sp.		24.67				44.88					98.63		16.82	AP	
Myrtaceae type 1	0.04						0.04				99.88		10.00	IIP	
Sapindaceae															
<i>Serjania pernambucensis</i> Radlk.						5.12							0.51	OIP	
Verbenaceae															
<i>Lippia</i> sp.									49.88				4.99	IIP	
Solanaceae															
<i>Solanum</i> sp.	31.98										0.34		3.23	IIP	
Solanaceae type 1												0.12	0.01	OIP	
Unidentified types															
NI1	17.76						0.49		0.06		0.34		1.87	OIP	
NI2		0.21	0.47			0.24	0.84		0.06		0.34		0.22	OIP	
NI3			0.16								0.34		0.05	OIP	
Total	100	100	100	0	0	100	100	100	100	100	100	100	100		

Note: AP, accessory pollen (15% < FR < 45%); IIP, important isolated pollen (3% < FR < 15%); OIP, occasional isolated pollen (FR < 3%); NI, not identified.

to Louveaux et al. (1978). The frequency classes include dominant pollen (DP, $FR > 45\%$), accessory pollen (AP, $15\% < FR < 45\%$), important isolated pollen (IIP, $3\% < FR < 15\%$) and occasional isolated pollen (OIP, $FR < 3\%$).

The similarity between the pollen samples of both studied bee species was obtained using the Sørensen similarity index, expressed by the formula $C_s = 2c / (s_1 + s_2)$, where s_1 is the amount of pollen collected by species 1, s_2 is the amount of pollen collected by species 2 and c is the amount of pollen collected by both species (Sørensen 1948).

Results

Pollen resources stored by Melipona scutellaris

Analysing the pollen collected by individuals of the species *Melipona scutellaris* revealed the occurrence of 15 pollen types identified from five plant families and three that were unidentified (Table I). The pollen from *Mimosa arenosa* (22.79%) (Fabaceae, Mimosoidae) and *Eucalyptus* sp. (23.73%) (Myrtaceae) were found most frequently during the study, and the richness of pollen was similar among the studied months (Table I). The highest pollen richness and relative frequency (41.55%) belonged to Fabaceae (Mimosoidae), which featured pollen from seven species in the samples analysed: *Acacia bahiensis*, *Acacia* sp., *Mimosa arenosa*, *Mimosa caesalpiniaefolia*, *Mimosa pudica*, *Mimosa* sp. 1 and *Mimosa* sp. 2 (Table I). Pollen from Myrtaceae, represented by *Eucalyptus* sp., *Psidium* sp. and Myrtaceae 1, was also observed throughout the sampling period, particularly pollen from *Eucalyptus* sp., which dominated a large portion of the monthly assessments.

In general, DP was not observed in the pollen samples stored by *Melipona scutellaris*; instead, AP predominated (46.52% of the analysed samples), represented by pollen from *Mimosa arenosa* and *Eucalyptus* sp. (Table I); followed by IIP (43.63%). The OIP class represented 9.85% of the samples.

Pollen resources stored by Melipona quadrifasciata anthidioides

The stored pollen samples from *Melipona quadrifasciata anthidioides* revealed 13 identified pollen types distributed across five families. Three pollen types were unidentified. Pollen from *Mimosa arenosa* (24.27%), *Eucalyptus* sp. (16.82%) and *Mimosa pudica* (13.84%) were the most common among the samples. The greatest pollen richness occurred in September. During June and July, there was no trace of pollen collected by bee populations of this species (Table II).

Pollen belonging to Fabaceae (Mimosoidae: *Acacia bahiensis*, *Mimosa arenosa*, *M. caesalpiniaefolia*, *M. pudica*, *Mimosa* sp., *Acacia* sp.) and to Myrtaceae (*Eugenia uniflora*, *Eucalyptus* sp. and Myrtaceae type 1) were the most common, corresponding to 55.59% and 33.55% of the samples, respectively (Table II), amounting to 89.14% of the total pollen samples. The distribution of different classes of abundance over the period of study made it possible to classify the pollen as IIP (53.83%), AP (41.09%) or OIP (5.2%).

Collected pollen similarity between species

The largest relative frequency of pollen in the samples of both species occurred in the dry season, from September to December 2012. This occurred because the reduced rainfall and humidity, the higher temperatures (Agrimtempo 2015) and the increased number of flourishing plant species restored the pollen load. *Melipona quadrifasciata anthidioides* and *M. scutellaris* collected 16 and 17 pollen types, respectively. Of these, 14 pollen types were collected by both species. Two types were collected exclusively by *M. quadrifasciata anthidioides* (pollen from *Eugenia uniflora* and *Lippia* sp.) and three exclusively by *M. scutellaris* (*Psidium* sp., *Croton heliotropiifolius* and Sapindaceae type 1) (Table III). The Sørensen similarity index between species was 0.79, a value meaning the bee species shared the pollen collection pasture at an overlapping rate of approximately 80% (Table III).

In June and July, it was not possible to estimate the similarity between the two species because *Melipona quadrifasciata anthidioides* stored no pollen during this period, which prevented the completion of the sample collection. The highest similarity values were found in April and August of 2012 ($C_s = 0.67$), and the lowest index was observed in October 2012 ($C_s = 0.17$), when the pollen harvested did not overlap between the two species.

Over the period of study, the diversity of pollen types collected by *Melipona scutellaris* was consistently greater than that collected by *M. quadrifasciata anthidioides*, except in September and November 2012. During the months in which both species collected pollen grains, the ratio of the amount of pollen stored by *M. scutellaris* and *M. quadrifasciata anthidioides* was highest in February 2013 ($7/2 = 3.5$) and lowest in November 2012 ($3/5 = 0.6$).

The most abundant pollen in the samples was *Mimosa arenosa*, with 10 685 counted pollen grains (46.36% found in the samples of *Melipona quadrifasciata anthidioides* and 53.64% in samples of *Melipona scutellaris*). Both species stored this pollen in all the study months, revealing it as an important food

Table III. Similarity between *Melipona quadrfasciata anthidioides* and *M. scutellaris* as pollen types found in samples stored in the colonies.

Pollen types	2012												2013												Presence																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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Note: Mq = *Melipona quadrfasciata anthidioides*; Ms = *M. scutellaris*.

source for both species. The second most abundant pollen in the samples was *Eucalyptus*, with 4258 counted pollen grains (29.99% found in the samples of *Melipona quadrifasciata anthidioides* and 53.64% in samples of *Melipona scutellaris*). The number of families to which visited plants belonged was the same (six families) for both *Melipona scutellaris* and *Melipona quadrifasciata anthidioides*. Of these, four families were visited by both species.

Discussion

The high frequencies of pollen from *Mimosa arenosa* (22.79%) (Fabaceae, Mimosoidae) and *Eucalyptus* sp. (23.73%) (Myrtaceae) demonstrates the affinity of *Melipona scutellaris* for these pollen sources. These frequencies can be attributed to foraging economy (Imperatriz-Fonseca et al. 1993), availability of food in the foraging radius of this species (Novais et al. 2015) and probably also to the almost-annual flowering of these species. In addition, these plants are cultivated (*Eucalyptus* sp.), ruderal (*Mimosa arenosa*), and large amounts of their pollen grains are present in the honey (Barth 1989) produced by the two bee species.

The clear affinity of *Melipona scutellaris* for the pollen of *Mimosa arenosa* and *Eucalyptus* sp. elucidated in the present study is consistent with the results of another palynological study, which demonstrated that *Melipona scutellaris* prefers a range of plant species, with Myrtaceae and Fabaceae among the six most important families (Ramalho et al. 2007). Regarding the high diversity and frequency of Fabaceae pollen observed in this study, the floral affinity of *Melipona scutellaris* for these plants is a behaviour reported in the specialised literature (Carvalho et al. 2001).

Five families were represented in the spectrum of pollen stored by *Melipona scutellaris*. The richness of pollen during the study period reveals the generalist behaviour of *M. scutellaris*. This seems to be standard behaviour for eusocial bees that gather and store food throughout the year for colony maintenance and that are perennial, populous and characterised by continuous offspring production (Serra et al. 2012) – even in periods of low food availability (Michener 2007). The contribution of *Eucalyptus* to the pollen stocks for several species of *Melipona* has already been reported in works such as Hilgert-Moreira et al. (2014), Luz et al. (2011) and Serra et al. (2012).

Serra et al. (2012) evaluated the pollen resources used by *Melipona capixaba* Moure et Camargo in the altitude forest and found *Eucalyptus* pollen in samples collected throughout the year. The Myrtaceae is among the most important pollen sources from the genus *Melipona* (Ramalho et al. 1990; Carvalho et al.

2001; Antonini et al. 2006). The predominance of Myrtaceae may be related to the fact that they bloom in masse over several days, resulting in energy savings by *Melipona* because they can employ the same harvest and flower handling mechanisms (Ramalho et al. 1994; Michener 2007). However, this does not indicate a reduced total effort during collection because the bees simply visit a larger number of flowers in search of pollen (Novais et al. 2015). Flowers of several species of Myrtaceae present longitudinal anthers from which bees extract pollen by rapidly vibrating their flight muscles (a technique called ‘buzz pollination’), which is a specialised behaviour of *Melipona* (Michener 2007). In addition, an individual forager tends to revisit the same food source after a previously successful visit (Biesmeijer et al. 1998), which influences the colony foraging behaviour (Hofstede & Sommeijer 2006).

The results of the analyses of stored pollen samples of *Melipona quadrifasciata anthidioides* were similar to studies carried out by Carvalho et al. (2001) in Bahia State and by Modro et al. (2011) in São Paulo with bees of the Apidae family. The low monthly variability in the diversity of pollen collected in the present study demonstrates the specificity of *M. quadrifasciata anthidioides* for certain species belonging to the subfamily Mimosoidae, Fabaceae. This affinity was clarified during the pollen spectrum investigation. Oliveira-Abreu et al. (2014) discusses the pollen collection selectivity of *M. quadrifasciata anthidioides* for many local plant species, as well as seasonal specificity for collecting resources.

According to Zanella and Martins (2003), larger numbers of plant species flower during the dry season; periods of increased flowering diversity do not coincide with the periods of heavy rains. The rainy season is followed by a period of plant vegetative development in preparation for subsequent flower production. According to Agritempo (2015), the temperature in the study area during June and July was lower (between 18 °C and 27 °C) and the humidity was higher (between 53% and 73%) compared to the other months of the year. These temperature and humidity factors may have influenced the shortage of stored pollen of *Melipona quadrifasciata anthidioides*.

Four plant families were visited by both *Melipona scutellaris* and *M. quadrifasciata anthidioides*, showing the high similarity between these two species and their specialisations in the use of trophic resources (especially pollen) from some families – primarily species of Myrtaceae, Fabaceae and Solanaceae. These three families present the richest pollen for *M. capixaba* (Luz et al. 2011). Santana et al. (2011), who carried out a pollen analysis in social bee colonies, found that Fabaceae and some species

of the subfamily Mimosoideae were among the most represented pollen types.

Ramalho et al. (2007) conducted a comparative analysis of the dynamics of various pollen sources between *Apis mellifera* L. and *Melipona scutellaris* in Cruz das Almas, Salvador and Alagoinhas, Bahia. The authors concluded that the main floral sources used by both species in the three locations belonged to the Myrtaceae and Fabaceae (Mimosoidae), corroborating the results of this study. Carvalho et al. (2001) found the same results in a study of pollen collection in areas where *M. scutellaris* occurs naturally.

Ensuring the availability of plant diversity related to the pollen spectra of both these species of *Melipona* is important to ensure that they can harvest different food sources and, consequently, obtain a well-balanced diet to supply their nutritional needs (Luz et al. 2011).

Conclusion

The results of this study demonstrate the niche similarity of pollen pasture between *Melipona scutellaris* and *M. quadrifasciata anthidioides* as well as their specialisations, which may arise either from bee affinity in gathering pollen from plant species belonging to certain families or simply from the abundance of these plant species in the region. This niche similarity interpretation is supported by the fact that although each bee species collected pollen belonging to six plant families, confirming the generalist habit of *Melipona*, the pollen from four of these families was represented in the samples stored by both species of bees. These results suggest that conservation efforts should be directed towards forest recovery to ensure that the species and plant families most visited by bees to obtain resources are represented, particularly the native species. In human-occupied areas such as the study region, meliponiculture farmers must promote the planting of the most-visited species, including exotic species, to the extent permissible by national environmental legislation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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