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Bee Life in the City: an Analysis of the Pollen Provisions of *Centris* (*Centris*) *flavifrons* (Centridini) in an Urban Area

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Abstract

Due to deforestation and fragmentation of ecosystems, the management of bee populations targeting pollination services is increasingly urgent. Because urban environments are stressful, the dietary knowledge in such areas can help to cope with this issue in the near future. Using palynological analysis the floral resources used by *Centris flavifrons*, an important pollinator of crops and native plants was studied in an urban area. *Byrsonima sericea* type, *Solanum paniculatum* type, *Cestrum* type, and *Myrcia* type 1 together accounted for more than 93% of pollen grains foraged by females. It is noteworthy that this bee population depends on few plant species both for pollen and for oil. Furthermore, females showed flexibility to replace the primary pollen source in different breeding seasons, as well as one female could provision different cells in the same nest with different predominant pollen types. The importance of wastelands and natural areasis highlighted for keeping bee populations in urban areas.

Introduction

Palynological analysis is an important tool in the study of bee food resources, and in the determination of the floral resources used by bees (Dórea et al., 2009; 2010a; 2010b; 2013; Santos et al., 2013; Cruz et al., 2015). The pollen stored in nests provides many data on bee foraging and help to fill the knowledge gap concerning the floral resources needed to maintain bee populations (Dórea et al., 2013). This is particularly important for bee populations that inhabit urban areas where food resources are generally sparse and its diversity can be quite limited. Although most of the studies indicate a decrease in species richness and abundance of pollinators (e.g. Zanette et al., 2005) even in the cities a substantial number of bee species can be found (Cane et al., 2006; Banaszak-Cibicka & Zmihorski, 2011). With the continuous and rapid growth of cities, ecosystems depend increasingly on urban development patterns (Alberti, 2005). Amid discussions about loss of biodiversity, urban areas are seen with a potential to promote diversity as a result of designing and planning the cities to foster interaction between people and nature (Miller, 2005).

Centris (*Centris*) *flavifrons* (Fabricius) has mainly a Neotropical distribution, occurring also in the Nearctic region in Sinaloa, Mexico (Moure et al., 2012) and is an important pollinator of *Malpighia emarginata* L., called West Indian cherry, and others agricultural crops, for example *Passiflora alata* Curtis (Gaglianone et al., 2010; Vilhena et al., 2012). It has also an important role in native plant species pollination in the Neotropical region, especially of Malpighiaceae, e.g. *Byrsonima sericea* DC. (Teixeira & Machado, 2000), *B. gardneriana* A. Juss. (Bezerra et al., 2009) and *B. cydoniifolia* Mart. (Sazan et al., 2014).

The nesting biology and ecology of this ground-nesting solitary bee species were initially studied by Vinson and Frankie (1988), Rêgo et al. (2006), Gonzalez et al. (2007) and,

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recently in a more detailed study by Martins et al. (2014). At open areas of coastal savanna in the Atlantic Forest dominium, including urban areas in the Northeastern coast of Brazil, females nest mainly during the dry season, associated to flowering that occurs mainly during the dry period and at the beginning of the rainy season (Lima et al., 2008), and exhibit notable flexibility for time each nest is active and the number of brood cells produced (Martins et al., 2014). Females were observed building until three nests, however the period of female activity in each nest, ranged from 2 to19 days (mean=6.9±4.2, n=141) and, consequently, the number of cells constructed per nest by each female ranged from 2 to 12 (Martins et al., 2014). Furthermore, a previous study suggested that *C. flavifrons* presents two to three generations per year (Martins et al., 2014).

Considering the lack of knowledge about the diet of this pollinator, and the existence of an aggregation of *C. flavifrons* resident in an urban area (Martins et al., 2014),where fragmented vegetation areas were available, the trophic resources exploited by this population were investigated.

Material and Methods

Study area

The study area was located in an urban area of João Pessoa city (7°9′11″ S; 34°50′28″ W), Paraiba State, Northeastern Brazil. The climate is hot and humid, tropical (As’, accordingly to Köppen classification), with annual average temperatures of 26 °C. The rainy season lasts from March to August, and the average annual rainfall is 1,700 mm (Lima & Heckendorff, 1985).

The nest aggregation of *C. flavifrons* was established in a home garden (642.46 m^2 total area) planted with fruit trees (*Malpighia emarginata* DC., *Mangifera indica* L., *Anacardium occidentale* L., *Spondias purpurea* L., *Eugenia uniflora* L., *Syzygium malaccense* (L.) Merr. & L.M. Perry, *Psidiumguajava* L., *Citrus limon* (L.) Osbeck, *Citrus sinensis* (L.) Osbeck, *Cocos nucifera* L., *Carica papaya* L., and *Morus* sp.) and grass. The garden had some small areas with bare soil: 22 bare ground circles, approximately 1 m in diameter, surrounding each of the orchard trees, and a narrow strip of land along the walls of the garden (20 cm×167.6 m) used for nesting by the female bees (see more details in Martins et al. (2014). Surrounding the garden area with the aggregation of nests there are some vacant lots, and at about 1,200 m distant there is a fragment of a well conserved Atlantic Rainforest with 471 ha, and smaller forest fragments.

Pollen analysis

Twelve broodcells from six nests of *C. flavifrons* from three different generations (Nov 2011, Feb 2012, and Nov/Dec 2012) were randomly sampled in this aggregation and selected to the pollen analysis of the provisions. Nest content of each sample (a cell) was treated with the acetolysis method (Erdtman, 1960).

After the chemical treatment, the samples from each cell were mounted in glycerine jelly on five slides. The pollen types were identified using the palynological catalogs (Roubik & Moreno, 1991;Carreira et al., 1996; Carreira& Barth, 2003; Melhem et al., 2003; Silva et al., 2010) and the reference slide collection of the palynotheca of Plant Micromorphology Laboratory from Universidade Estadual de Feira de Santana (LAMIV/UEFS). In addition, lists of floristic surveys surrounding the study area were used in order to have knowledge of the local flora (Barbosa, 2008; Amazonas & Barbosa, 2011; Gadelha-Neto & Barbosa, 2012).

At least 1,000 pollen grains were counted per sample (and at least 200 pollen grains per slide) for the quantitative analysis, following Vergeron (1964). We calculated the occurrence frequency of each pollen type in each sampled brood cell, as well as the averages of the frequencies of each pollen type among all the samples (according with Villanueva-Gutiérrez & Roubik, 2004).

Results

From the pollen contents of twelve brood cells from *C. flavifrons* nests it was elaborated a pollen spectrum (Fig 1) containing 16 pollen types. Fifteen pollen types were related to eight plant families and only one could not be identified (Table 1). *Byrsonima sericea* type, *Solanum paniculatum* type, *Cestrum* type, and *Myrcia* type 1 together accounted for more than 93% of pollen grains foraged by the females of *C. flavifrons*.Thus, the plant species related to these pollen types were the main protein sources in the diet of this bee population.

Myrtaceae (4 pollen types) and Solanaceae (3) were the families with the highest number of different pollen types. Other families ranged from one to two different types of pollen grains. However, Malpighiaceae was the only family represented in all samples. Moreover, it was the family with the second most foraged pollen type (*Byrsonima sericea*) amounting 30.44% of the total number of pollen grains counted. The type *B. sericea* was identified in eleven of the twelve samples analyzed, ranging from very low percentages (0.10%) to nearly the entire sample (97.07%).

Although they have been identified in nearly half of the samples, *Cestrum* (23.11%) and *Solanum paniculatum* (30.75%) types had high percentages in the total pollen grains counted. In only one sample, no pollen grains of Solanaceae were present.

Myrtaceae was the most represented family in number of distinct pollen types, however they were present in only six samples. In addition, in one sample a high percentage (97.30%) of *Myrcia* type 1 was found. Among the total of 16 pollen types, ten pollen types showed percentages below 10% in the samples analyzed. Six types of pollen grains were exclusively found in only one sample and at least five have been identified in six or more samples.

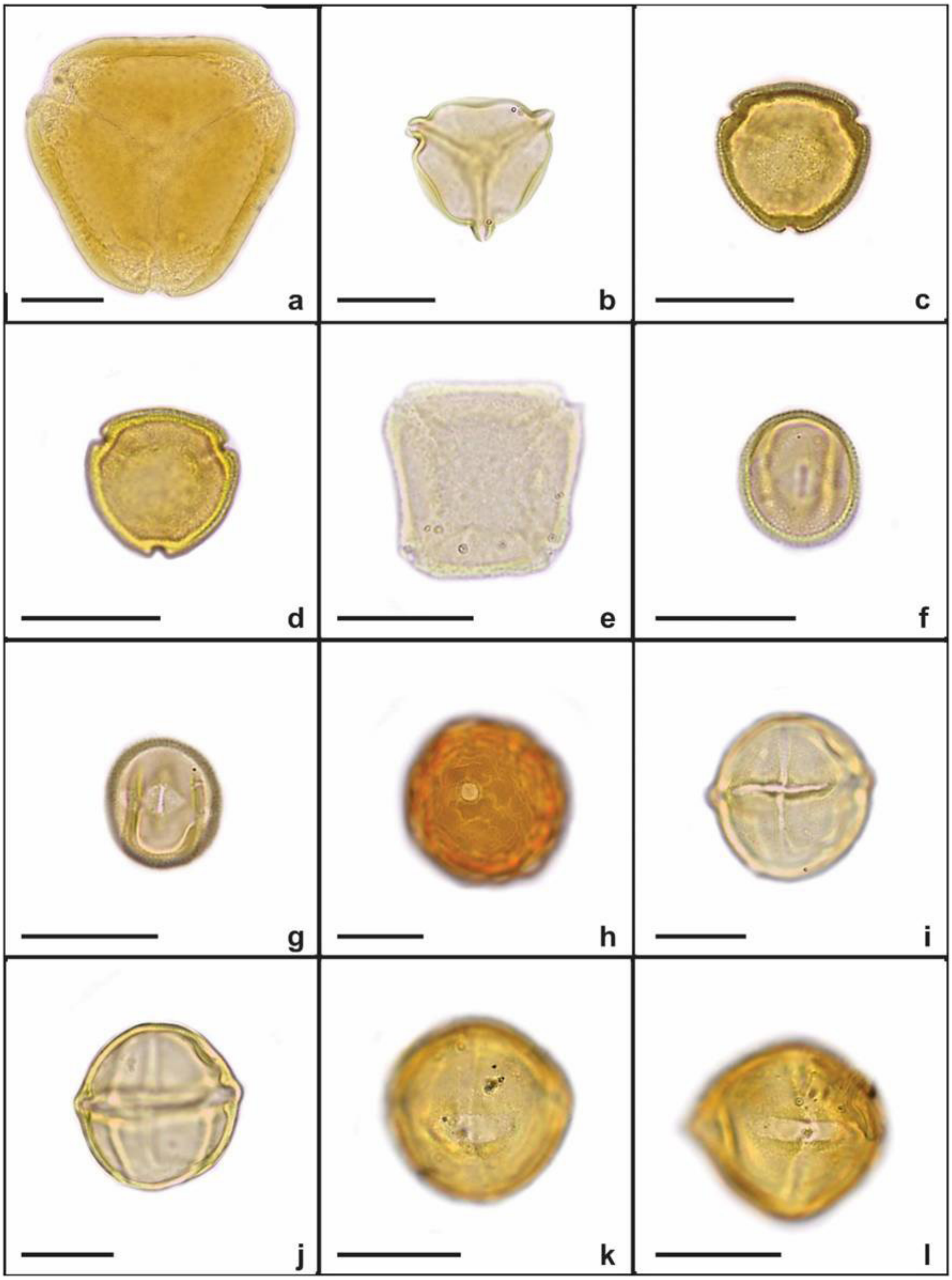

Fig 1. Pollen types identified in nests of *Centris* (*Centris*) *flavifrons* in an urban area. Fabaceae: a. *Dioclea*. Lythraceae: b. *Cuphea*. Myrtaceae: c-d. *Myrcia* type 1. e. *Psidium*. Malpighiaceae: f-g. *Byrsonima sericea*. h. *Stigmaphyllum blanchetii*. Solancaceae: i-j. *Cestrum*. k-l. *Solanum paniculatum*.

It is noteworthy that brood cells provisioned in the same period by different females could contain a high percentage of the same pollen type (cells from nests 1 to 4 with more than 90% of *Solanum paniculatum* type, Fig 2), and cells provisioned by the one female in the same nest showed, or not, a high percentage of the same pollen type (cells 30 and 32 from nest 5 contained 82 to 90% of *Byrsonima sericea* type; while in cells 14 and 31, from the same nest, predominated 97.3% of *Myrcia* type 1 and 82.6% of *Cestrum* type, respectively; a similar pattern occurred in cells from nest 6, Table 1, Fig 2).

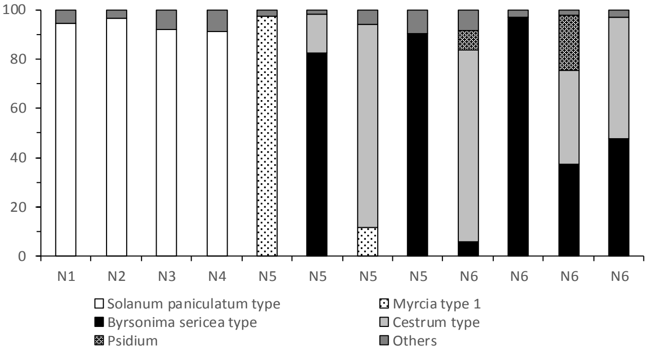

Fig 2. Proportion (%) of different pollen types in brood cells from six nests (N1 to N6) of *Centris* (*Centris*) *flavifrons*. Each column represents one cell, and the sequence of cells is the same presented in Table1.

Table 1. Frequency of pollen types (%), recorded in brood cells/nests of *Centris (Centris) flavifrons* within an urban area in the municipality of João Pessoa, Paraíba State, Brazil. Und= undetermined.

	Nov 2011	Nov 2011	Nov 2011	Nov 2011	Feb 2012	Feb 2012	Feb 2012	Feb 2012	Nov-Dec 2012	Nov-Dec 2012	Nov-Dec 2012	Nov-Dec 2012
CELLCODE/$^{\text{NEST CODE}}$	4^{1}	6^{2}	15^{3}	11^{4}	14^{5}	30^{5}	31^{5}	32^{5}	17^{6}	24^{6}	25^{6}	26^{6}
POLLEN TYPE/SAMPLES	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Leg. Caesalpinioideae												
Chamaecrista type					0.10							
Leg. Papilionoideae												
Clitoria racemosa type							0.09		0.20			
Diocleatype		0.20	1.80	1.00					0.20		1.05	0.38
Lytraceae												
Cupheatype							2.15					
Malpighiaceae												
Byrsonima sericea type	1.70		0.50	0.10	0.30	82.45	0.43	90.34	5.77	97.07	37.36	47.71
Stigmaphyllon blanchetii type		0.10	0.50	1.10	0.20	1.69		0.19		0.29	1.14	2.39
Moraceae												
Brosimumtype							0.17					
Myrtaceae												
Myrciatype 1			0.10	0.30	97.30		11.44					
Myrciatype 2					2.10							
Myrciatype 3									4.89			
Psidium									8.12		22.15	
Solanaceae												
Cestrumtype						15.85	82.63		77.79	2.44	38.21	49.52
Solanum paniculatum type	94.50	96.50	91.90	91.30			3.10		2.64	0.10		
Solanumtype 2	3.80	3.10	5.20	6.20								
Urticaceae												
Laportea type		0.10							0.39	0.10	0.10	
Und. type								9.47				
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100

Discussion

The main feature of the *C. flavifrons* diet in this urban landscape is the high dependence on a few (four) species of plants as protein sources for rearing offspring, suggesting that this bee population has a narrow realized trophic niche. Based on literature data on floral resources available from plant species, *Chamaecrista* type, *Solanum* type, *Myrcia* types 2 and 3, *Psidium* type, *Brosimum* type and *Laporteaaestuans* type are probably related to other pollen sources (Machado & Lopes, 2006; Gressler et al., 2006; Pederneiras et al., 2011; Gaglioti et al., 2016), but they were little exploited by this population of *C. flavifrons* in the temporal window analyzed. Although *Stigmaphyllon* species are exploited for pollen by Centridini bees (Sigrist & Sazima, 2004), the frequency of occurrence *S. blanchetii* type in our samples suggested that the related plant was exploited only by oil. *Cuphea* type is related to plant species that supply nectar (Cavalcanti & Graham, 2002), as also *Cestrum* type (Silva et al., 2003).

Similarly, in an agricultural area surrounded by savanna vegetation, *C. flavifrons* females collected pollen from different plant species, but they showed stronger interactions with only three species of Malpighiaceae and *Anacardium* sp. (Vilhena et al., 2012), which were more quantitatively represented in pollen loads of foraging females. Although the number of studies on the diet of this species is very low, there are clues that the pollen diet of the larvae tends to be based on a few plant species in different types of landscapes, such as natural vegetation (Gaglianone, 2003; Rego et al., 2006), crops surrounded by native vegetation (Vilhena et al., 2012) and urban areas (this study). A similar trend has been reported for other species of this genus, as *Centris flavofasciata* Friese and *Centris nitida* Smith also showed a high concentration of foraging for pollen in a few plant pecies (3 to 2 species) in tropical deciduous forest vegetation in Mexico (Quiroz-Garcia et al., 2001; Quiroz-Garcia & Arreguin-Sánches, 2006). Although many pollen types have been collected by the females in these studies, two Malpighiaceae species only accounted for most of foraged pollen.

Our findings also indicate that the breeding females of *C. flavifrons* have the flexibility to replace the primary pollen source in different breeding seasons, as indicated by the replacement of *S. paniculatum* in Nov/2011 season by *B. sericea* or by *Cestrum* type in the other two nesting seasons. Moreover, females of the same generation may focus the foraging for pollen in the same plant species (e.g. Nov/2011) or may use two or three different plant species (e.g. Feb/2012, Nov-Dec/2012), which suggests an adjustment of nesting females to the local availability of some preferred sources of pollen. Similarly, Rêgo et al. (2006) analyzed the pollen content of four cells from different nests of *C. flavifrons* and found that in each cell the most important pollen type was different. Furthermore, this flexibility is reinforced by the provisioning of different cells with different predominant pollen types by the same female in the same nest, observed in this study.

Our study also showed a low diversity of floral oil sources in the diet of this *C. flavifrons* population, an essential resource for Centridini nesting females, since it is used as material for lining the brood cells, nests waterproofing, and as larval food for many *Centris* species (Vinson & Frankie, 1988; Jesus & Garófalo, 2000; Aguiar & Gaglianone, 2003). Another population of *C. flavifrons* in a savanna vegetation also showed dependence on a single source of oil and pollen (*Byrsonima intermedia*), although several other Malpighiaceae species were available during the nesting season (Gaglianone, 2003).

Despite the dependence on a limited number of plant species, the reactivation of the aggregation for several consecutive years, and the production of several hundred nests by this species of bee in the same nest site (Martins et al., 2014) indicate that this urban landscape meets an adequate abundance of resources to support this population of *C. flavifrons*. To the maintenance of this bee population it is noteworthy the existence of Solanaceae species, used as a pollen source by this and other species of bees, that grow spontaneously in wastelands (vacant lots or unbuilt areas inside the city), as well as the proximity to areas where there are pioneer species, such as *Byrsonima sericea*, which can reach high densities and provide pollen and oil for the Centridini bees (Rosa & Ramalho, 2011; Aguiar, unpubl. data). Nevertheless, although *B. sericea* occurs in the urban matrix, it is more abundant in the close Atlantic Rainforest fragments 1,200 m distant, a distance that the medium-large sized *C. flavifrons* can fly (Zurbuchen et al., 2010) while *S. paniculatum* occurs abundantly in wastelands. These two species together accounted for 61% of the collected pollen.

Fragmentation, habitat degradation, and modern agricultural practices have been affecting the biota by the elimination of resources needed for successful reproduction such as nesting sites and pollen and nectar sources and are responsible for the decline of many bee species populations (Müller et al., 2006; Andrieu et al., 2009). Urban areas are an extreme case of habitat fragmentation; therefore, it is relevant to know the adaptations of the foraging behavior of pollinators in these areas (Andrieu et al., 2009). Nevertheless, bee species, according to ecological traits associated with nesting and dietary breadth, may respond differently to urban habitat fragmentation in the Arizona desert (Cane et al., 2006). For instance, oligolectic ground-nesting species were underrepresented in smaller fragments and less abundant in the smaller and older fragments, while decline of polilectic ground-nesting species was weak and insignificant, and cavity-nesting bees were overrepresented in the habitat fragments, probably due to enhanced nesting opportunities available in the urban matrix (Cane et al., 2006). Thus, this knowledge is likely to become crucial to conserve and manage populations of bees in the future. Our study shows the importance of wastelands and natural areas to *C. flavifrons*, and probably other bee species populations in urban areas. Wastelands are important areas for ruderal plant species that provide pollen and nectar to maintain crop pollinators in periods of floral resource shortage (Cruz & Martins, 2015). Furthermore, it has been suggested that a close neighborhood of nesting and foraging habitat within few hundred meters is crucial to maintaining populations of most bee species (Zurbuchen et al., 2010), which strengthens the proposal of maintaining these habitats in urban areas.

Concluding remarks

Using palynological analysis our study showed that the polilectic ground-nesting bee species *C. flavifrons* actually may rely on few plant species for brood rearing, and that females showed flexibility to replace the primary pollen source in different breeding seasons, or in the same season, probably according to availability and spatial distribution of pollen and oil sources. Four pollen types, *Byrsonima sericea* type, *Solanum paniculatum* type, *Cestrum* type, and *Myrcia* type 1 together accounted for most of the pollen grains (>90%) foraged by females. Thus, the plant species related to these pollen types are key pollen sources for the maintenance of this bee population.

Some plant species occur in a forest fragment relatively close to the study area but others grow in vacant lots inside the city, highlighting the importance of keeping natural areas and wastelands inside urban areas for keeping this, and likely other bee species and animals. In the future the planning of cities should consider the use of natural areas including areas that can be used for entertaining, human well-being, and at same time for keeping flora and fauna in general.

Therefore, more studies, especially long term approaches, arenecessary to understand the population dynamicsof *C. flavifrons* and its interactions with plant species in natural and urban areas.

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