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Small pollen grain volumes and sizes dominate the diet composition of three South American subtropical stingless bees

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Small pollen grain volumes and sizes dominate the diet composition of three South American subtropical stingless bees

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Abstract

The pollen preferences of three Meliponini species (*Geotrigona argentina*, *Scaptotrigona jujuyensis* and *Tetragonisca fiebrigi*) were studied in the Chaco forest of South America. Surprisingly, a large number of pollen types with small volumes and size were found in pollen masses stored in the 18 nests studied. Small pollen grains are commonly found in small flowers with short pistils, a flower morphology adapted to the pollination by these small- to medium-sized bees. The prevalence of grains with small volumes may be due to the preference of Meliponini bees for small flowers with short pistils, supporting a previously suggested hypothesis of diffuse co-evolution between mass-flowering trees and these tiny bees in Neotropical habitats. However, the opportunistic foraging habit of these generalist bees would suggest that the large number of grains with small size is due to their greater availability in the Chaco forest nearby the nests. This study also provides measurements and descriptions of pollen cytoplasmic shapes as well as a new classification of pollen volumes based from measurements of the five pollen size categories using non-acetolyzed grains.

Keywords: *corbiculate bee*, *cytoplasmic volume*, *Geotrigona argentina*, *opportunistic bee*, *pollen size category*, *Scaptotrigona jujuyensis*, *Tetragonisca fiebrigi*

The Meliponini bee fauna of the subtropical Dry Chaco forest of Argentina is composed of at least seven species (Arenas 2003; Vossler 2012; Roig-Alsina et al. 2013). To maintain their perennial colonies with hundreds to thousands of individuals and high rates of offspring, these bees have to forage large amounts of food from a great number of plants from diverse families (Roubik 1989; Ramalho et al. 2007).

It is well known that other corbiculate bees such as the widely studied *Apis mellifera* L. forage on a broad spectrum of plants. For instance, O'Rourke and Buchmann (1991) found that *A. mellifera* gathered similar amounts of large, medium and small sizes of pollen grains in a study carried out in an arid region of the Sonoran Desert in Arizona, but Biesmeijer et al. (1992) found that it foraged mainly on small pollen grains in Suriname. Unfortunately, few studies have recorded the volume and size of the pollen grains foraged by Meliponini bees to make comparisons.

For instance, it has been reported that the Brazilian *Trigona spinipes* (Fabricius) forage on a broad spectrum of pollen sizes from 10 to > 100 μm , while *A. mellifera* forage on a smaller range (10 to \leq 71 μm) in the same area (Cortopassi-Laurino & Ramalho 1988). Marques-Souza et al. (2002) reported that pollen grains of diverse sizes are equally present among the pollen gathered by the Brazilian Amazonian *Frieseomelitta*, *Melipona* and *Scaptotrigona*. In the Dry Chaco forest, Vossler et al. (2010) reported that *Geotrigona argentina* Camargo et Moure intensively foraged (\geq 5%) on pollen types of very small to medium volumes (from 65 to 34 782 μm^3).

In view of the polylectic specialization category of all members of this bee tribe, it is expected that the three subtropical Meliponini species studied forage on a wide spectrum of flower sizes having pollen grains from very small (< 10 μm and < 524 μm^3) to very large (> 100 μm and > 523 583 μm^3) both in size and

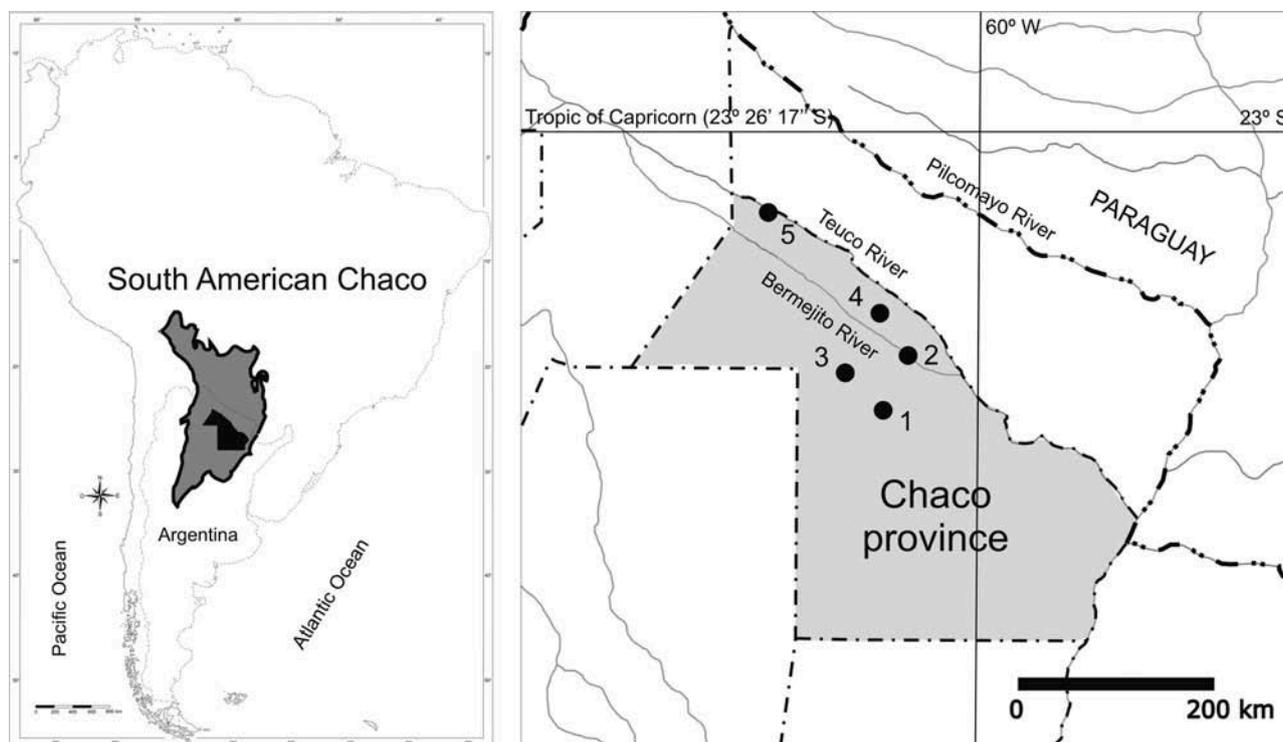


Figure 1. Map depicting the distribution of the subtropical Chaco forest of South America indicating the sites where nest pollen samples were obtained. 1, Juan José Castelli (25° 56' S; 60° 37' W); 2, Villa Río Bermejito (25° 37' S; 60° 15' W); 3, Miraflores (25° 29' S; 61° 01' W); 4, El Espinillo (25° 24' S; 60° 27' W); 5, El Sauzalito (24° 24' S; 61° 40' W).

volume. For this reason, the goals of this study were to establish the cytoplasmic volume and size categories of the pollen grains foraged by three species of Meliponini bees in the South American Chaco forest, particularly those intensively foraged, to detect a possible relationship between pollen size/volume and flower size, and to give possible explanations about why certain pollen grains are highly abundant in these colonies.

Material and methods

Dry Chaco forest vegetation

The vegetation units of the study area are 'Quebrachal' of three 'quebrachos' belonging to the central Chaco Forests, and 'Quebrachal' of two 'quebrachos' and 'Palosantales' of *Bulnesia sarmientoi* Lorentz ex Griseb. both belonging to the western Chaco Forests, *sensu* Prado (1993). The two former units are characterised by the dominance of *Schinopsis lorentzii* Engl. (Anacardiaceae), *Schinopsis balansae* Engl. and *Aspidosperma quebracho-blanco* Schldtl. (Apocynaceae), and the latter by *Bulnesia sarmientoi* Lorentz ex Griseb. (Zygophyllaceae). Other floristic elements are *Ziziphus mistol* Griseb. (Rhamnaceae), *Sideroxylon obtusifolium* (Roem. et Schult.) T.D. Penn. (Sapotaceae), several species of *Prosopis* (Fabaceae, Mimosoideae), *Caesal-*

pinia paraguariensis (D. Parodi) Burkart (Fabaceae, Caesalpinioideae), *Pterogyne nitens* Tul. (Fabaceae, Caesalpinioideae), *Tabebuia nodosa* (Griseb.) Griseb. (Bignoniaceae), *Aspidosperma triternatum* Rojas Acosta (Apocynaceae), *Trithrinax schizophylla* Drude (Arecaceae), *Castela coccinea* Griseb. (Simaroubaceae), *Achatocarpus praecox* Griseb. (Achatocarpaceae), *Celtis* spp. (Celtidaceae), *Schinus fasciculatus* (Griseb.) I.M. Johnst. var. *arenicola* (Hauman) F.A. Barkley (Anacardiaceae), *Capparis atamisquea* Kuntze (Capparidaceae), *Maytenus vitis-idaea* Griseb. (Celastraceae), *Moya spinosa* Griseb. (Celastraceae), *Mimosa detinens* Benth. (Fabaceae, Mimosoideae), *Stetsonia coryne* (Salm-Dyck) Britton et Rose (Cactaceae) and several species of *Capparis* (Capparidaceae) and *Acacia* (Fabaceae, Mimosoideae) (Prado 1993; Vossler 2013).

Pollen sampling and processing

Pollen masses (the pollen content of each cerumen pot) were extracted from one pot if it was complete or closed or a few if they were broken and/or opened. As each nest is an independent sample, pollen masses taken from the same nest were considered subsamples.

A total of 86 subsamples of pollen masses from ten nests were analysed for *Tetragonisca fiebrigi*

Table I. Pollen types intensively (xx) (in bold) and slightly (x) foraged by *Tetragonisca fiebrigi* (Tf), *Scaptotrigona jujuyensis* (Sj) and *Geotrigona argentina* (Ga).

Plant family	Pollen type	Bee species		
		Tf	Sj	Ga
Acanthaceae	<i>Justicia</i>	x	x	
Achatocarpaceae	<i>Achatocarpus praecox</i>	xx	x	x
Alismataceae	<i>Sagittaria montevidensis</i>	x	x	
Alismataceae/Limnocharitaceae	<i>Sagittaria montevidensis</i>/Hydrocleys			xx
Amaranthaceae	<i>Alternanthera</i>			x
Anacardiaceae	<i>Schinopsis</i>	xx	xx	x
Anacardiaceae	<i>Schinus fasciculatus</i>-<i>Schinopsis</i>?	xx	xx	
Apiaceae	Apiaceae	x	x	
Apocynaceae	Apocynaceae			x
Apocynaceae	<i>Aspidosperma</i>	x	x	
Arecaceae	<i>Trithrinax schizophylla</i>	xx	xx	
Asteraceae	<i>Parthenium hysterophorus</i>	xx	xx	x
Asteraceae	<i>Xanthium spinosum</i>	x	x	
Asteraceae	Astereae			x
Asteraceae	<i>Baccharis</i>	x	xx	
Asteraceae	<i>Tessaria dodoneifolia</i>	x	x	
Asteraceae	<i>Tessaria</i>			x
Asteraceae	Heliantheae	xx		
Asteraceae	<i>Holocheilus hieracioides</i>			x
Asteraceae	<i>Vernonia</i>	x	x	
Basellaceae	<i>Anredera cordifolia</i>			x
Bignoniaceae	<i>Fridericia dichotoma</i>	x	x	
Bignoniaceae	<i>Tabebuia</i>	xx		
Boraginaceae	Boraginaceae			x
Boraginaceae	<i>Heliotropium</i>			x
Bromeliaceae	Bromeliaceae	x		
Cactaceae	Cactaceae			x
Capparidaceae	<i>Capparis salicifolia</i>/C. tweediana	xx	x	
Capparidaceae	<i>Capparis speciosa</i>/C. tweediana	xx	x	
Capparidaceae	<i>Capparis atamisquea</i>	xx	x	
Capparidaceae	<i>Capparis retusa</i>	xx	x	
Capparidaceae	<i>Capparis</i>			xx
Celastraceae	<i>Maytenus vitis-idaea</i>/Moya spinosa	xx	x	xx
Celtidaceae	<i>Celtis</i>	xx	x	x
Chenopodiaceae	Chenopodiaceae	x		
Cyperaceae	<i>Eleocharis</i>			xx
Euphorbiaceae	<i>Croton</i>			xx
Euphorbiaceae	<i>Sapium haematospermum</i>	xx	x	
Fabaceae, Caesalpinioideae	<i>Pterogyne nitens</i>	xx	xx	
Fabaceae, Caesalpinioideae	<i>Caesalpinia paraguariensis</i>	xx	x	
Fabaceae, Caesalpinioideae	<i>Cercidium praecox</i>	xx	x	
Fabaceae, Caesalpinioideae	<i>Parkinsonia aculeata</i>	xx		x
Fabaceae, Mimosoideae	<i>Acacia</i> (polyad of 16 pollen grains)	x	x	x
Fabaceae, Mimosoideae	Ingeae 1 (monads from polyad)	x	x	
Fabaceae, Mimosoideae	Ingeae 2 (monads from polyad)	x	x	
Fabaceae, Mimosoideae	<i>Mimosa detinens</i>	x	xx	
Fabaceae, Mimosoideae	<i>Prosopis</i>	xx	x	xx
Fabaceae?	<i>Gleditsia</i>/Fabaceae?	xx	x	
Loranthaceae	<i>Struthanthus angustifolius</i>	x	x	xx
Loranthaceae	<i>Tripodanthus acutifolius</i>			x
Lythraceae	<i>Heimia salicifolia</i>	x	x	
Malpighiaceae	Malpighiaceae			x
Malpighiaceae	<i>Mascagnia brevifolia</i>	xx	x	
Malvaceae	Malvaceae	x	x	x
Menyanthaceae	<i>Nymphoides indica</i>			x
Nyctaginaceae	<i>Boerhavia diffusa</i>			x
Nyctaginaceae	<i>Pisonia zapallo</i>			x
Olacaceae	<i>Ximena americana</i>	x	x	
Onagraceae	<i>Ludwigia</i>			x

(Continued)

Table I. (Continued).

Plant family	Pollen type	Bee species		
		Tf	Sj	Ga
Polygonaceae	<i>Polygonum</i>	x	x	
Polygonaceae	<i>Ruprechtia triflora</i>	x		x
Portulacaceae	<i>Portulaca</i>			x
Rhamnaceae	<i>Ziziphus mistol</i>	xx	x	xx
Santalaceae	<i>Acanthosyris falcata</i>	x	x	
Sapindaceae	<i>Urvillea chacöensis</i>	x	x	
Sapotaceae	<i>Sideroxylon obtusifolium</i>	xx	x	x
Scrophulariaceae	<i>Scoparia</i>			x
Simaroubaceae	<i>Castela coccinea</i>	x	x	xx
Typhaceae	<i>Typha</i>			x
Ulmaceae	<i>Phyllostylon rhamnoides</i>	xx		
Verbenaceae	<i>Phyla reptans</i>	x	x	x
Verbenaceae	<i>Verbena</i>			x
Zygophyllaceae	<i>Bulnesia sarmientoi</i>			x
?	Tetrad	x	x	
?	Unidentified 1	x	x	
?	Unidentified 2	x	x	
?	Unidentified 3			x
?	Unidentified 4			x
	Number of pollen types $\leq 10\%$ (x)	28	38	32
	Number of pollen types $> 10\%$ (xx)	24	7	9
	Total number of pollen types per bee species	52	45	41

Note: The number of foraged pollen types varied among these species. These three bee species foraged intensively on a total of 32 pollen types.

(Schwarz), 18 subsamples from four nests for *Scaptotrigona jujuyensis* (Schrottky), and 63 from four nests for *Geotrigona argentina* (Vossler et al. 2010; Vossler 2013). Pollen types were classified in two categories according to their abundance: $\leq 10\%$ and $> 10\%$ (Table I) following Ramalho et al. (1985), Cortopassi-Laurino and Ramalho (1988), and Ramalho (1990). A total of 32 pollen types were intensively foraged ($> 10\%$) by the three Meliponini species studied. *Tetragonisca fiebrigi* intensively foraged on 24 out of the 52 foraged pollen types, *S. jujuyensis* on seven out of 45 and *G. argentina* on nine out of 41 (Table I). From the 78 pollen types identified in all subsamples, 37 of them were taken at random for both diameter and volume measurement.

Pollen type nomenclature follows the recommendations of Birks (1973), Birks and Birks (1980), Joosten and De Klerk (2002), and De Klerk and Joosten (2007). In most cases, pollen volumes and size were measured from reference pollen and therefore authority was not deleted.

The reference pollen collection was made from flower buds of plant species collected in various localities from the Chaco province of Argentina (Juan José Castelli [25° 56' S; 60° 37' W], Villa Río Bermejito [25° 37' S; 60° 15' W], El Espinillo [25° 24' S; 60° 27' W], Miraflores [25° 29' S; 61° 01' W] and El Sauzalito [24° 24' S; 61° 40' W]; Figure 1).

The plant specimens were pressed, dried, identified by the author and deposited in the herbaria of the Museo de La Plata (LP), La Plata, and the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (BA), Buenos Aires, Argentina. Plant nomenclature follows Zuloaga et al. (2008). Fresh pollen grains from flowers were dyed using fuchsin (but not acetolysed), mounted on slides using a glycerine-gelatine mixture and measured under a Nikon Eclipse E200 light microscope at 1000 \times magnification. When pollen from flowers was not available, pollen grains from the colonies were measured. Bees were identified by Arturo Roig-Alsina and deposited in the Entomology Collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'.

Categories of volume and size of pollen grains

Ten to 15 measurements of both polar and equatorial axes of pollen grain cytoplasm per each reference plant were taken. The measurements were from equatorial view for spherical and ellipsoidal shapes, and from two different views for prism shapes.

The pollen size categories were taken from Wodehouse (1935) and Hesse et al. (2009), being very small ($< 10 \mu\text{m}$), small (10–25 μm), medium (26–50 μm), large (51–100 μm) and very large ($> 100 \mu\text{m}$). To allocate a particular pollen type to

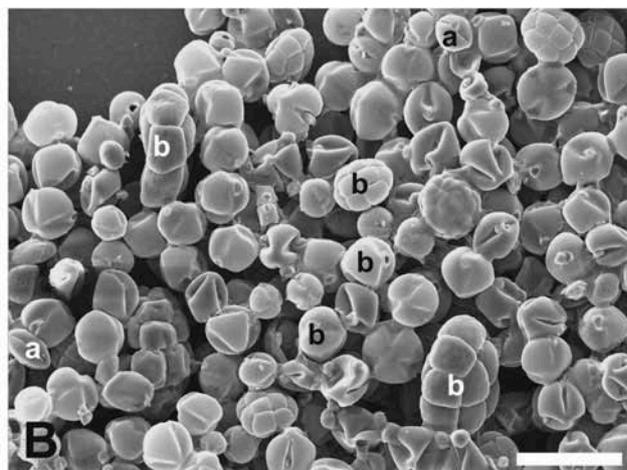
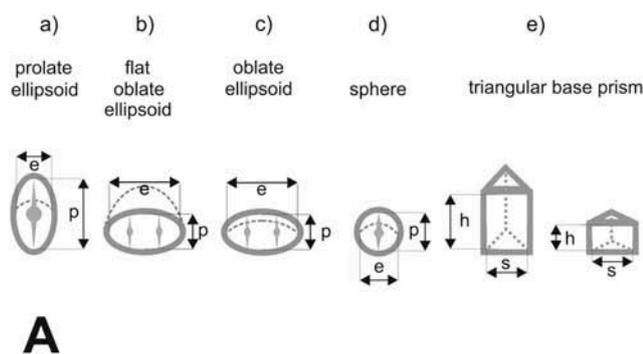


Figure 2. **A.** Pollen shapes (a–e) found in the pollen samples studied. Location of axis e and p (of ellipsoidal and spherical shapes) and h and s (of prismatic shape) is provided. **B.** Scanning photo-micrograph of grains found in pollen masses of Meliponini bees, showing different shapes and sizes. Scale bar – 50 μm .

each size category, the longest value of any of the two cytoplasmic diameters was used to establish the categories of whole pollen grains (cytoplasm plus walls), as suggested by Hesse et al. (2009).

To estimate the cytoplasmic volume, pollen grains were compared to similar geometric shapes: sphere, ellipsoid and triangular base prism. Based on the size classes, five pollen volume categories are used in the present study: very small volume ($< 524 \mu\text{m}^3$), small volume ($524\text{--}8181 \mu\text{m}^3$), medium volume ($8182\text{--}65\,448 \mu\text{m}^3$), large volume ($65\,449\text{--}523\,583 \mu\text{m}^3$) and very large volume ($> 523\,583 \mu\text{m}^3$). The calculations of volume categories are based on spherical pollen grains.

Pollen shapes

All shapes found in the samples were identified and measured in equatorial view as follows (see Figure 2A).

Prolate ellipsoid (and prolate ellipsoid polyads) (Figure 2A, B). — Main axis p (polar axis) and two minor axis e of the same length (equatorial axes; e.g. *Pterogyne nitens* and *Mimosa detinens* polyad). The formula is $1/6 \pi p e^2$. In the same way, the axis p of the prolate ellipsoid polyad was considered the axis of greater length, while e the minor axes.

Flat oblate ellipsoid (and flat polyads) (Figure 2A, B). — Minor axis p (polar axis) and two axes e (equatorial axes), they can be of the same length (e_2 ; e.g. *Acacia praecox* Griseb. and *Acacia* sp. polyads), or of different lengths (e_1 and e_2 ; e.g. polyad of *Enterolobium contortisiliquum* (Vell.) Morong). The formulae are $1/6 \pi p e^2$ or $1/6 \pi p e_1 e_2$, respectively. In flat

polyads, their thickness (corresponding to the minor axis) was considered as p axis, while both other major axis (height and width of the polyad) were considered as e axes (or e_1 and e_2 if they are different in length).

Oblate ellipsoid (Figure 2A). — Similar to prolate ellipsoid shape with a rotation of 180° . The main axis e (long equatorial axis) and two axes p of the same length (polar axis and short equatorial axis; e.g. *Salvia*). The formula is $1/6 \pi e p^2$.

Sphere (Figure 2A). — A polar axis p and an equatorial axis e of approximately the same length (e.g. *Polygonum*). The ratio between the mean values of these two axes must be within the 0.99 to 1.01 range. The formula is $1/6 \pi p^3$. In cases where spherical and prolate or flat oblate ellipsoidal pollen grains were found in the same pollen type, the prolate ellipsoid formula was applied (Figure 2A).

Triangular base prism (Figure 2A). — Being s any of the base sides and h the height of the prism (O'Rourke & Buchmann 1991; e.g. *Acanthosyris falcata* Griseb). As both values could not be measured in the same grain, s and h were measured in different grains. The formula is $1/4 s^2 h \sqrt{3}$.

Other shapes such as cubic shapes or those found in heteropolar pollen grains were not present among the pollen grains foraged by Meliponini bees from the Dry Chaco forest. Although the oblate ellipsoid shape was not found in the samples, it was described to avoid mistakes in volume calculations as it is very similar to the prolate ellipsoid shape. However, in cases where the oblate ellipsoid shape is present, its volume should be calculated according to Figure 2A

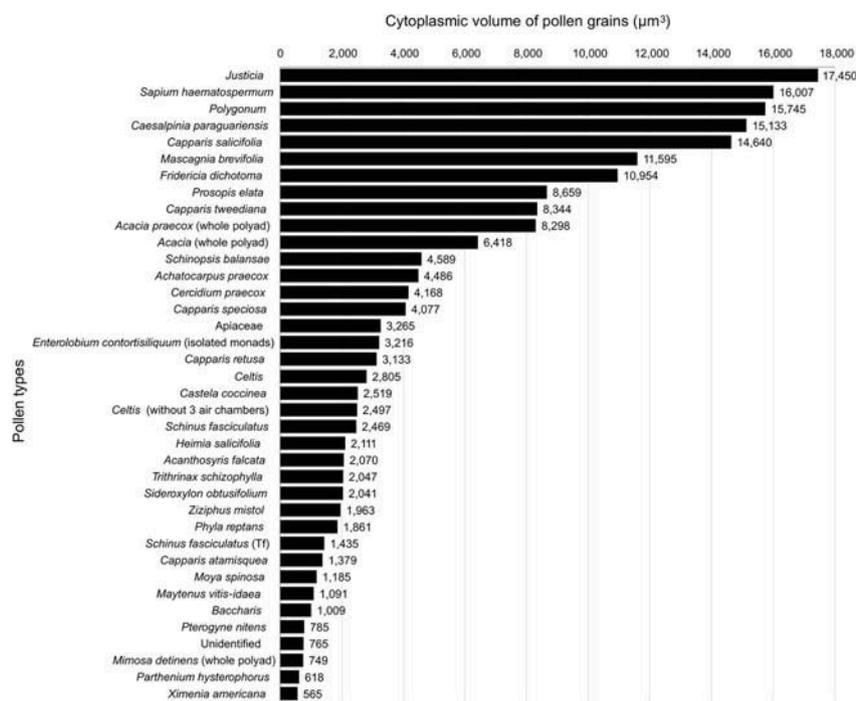


Figure 3. Volume (in μm^3) of 37 pollen types of melittophilous plant species of the subtropical Dry Chaco forest, in decreasing order. Volume values ranged from $565 \mu\text{m}^3$ for *Ximenesia americana* (Olacaceae) to $17\,450 \mu\text{m}^3$ for *Justicia* (Acanthaceae).

(c). In the same way, *Enterolobium contortisiliquum* (Vell.) Morong was not found among pollen samples as a whole polyad, but only as isolated monads. However, due to the fact that this acalymmate polyad could be broken during foraging or palynological processing, the formula to describe flat polyads is given.

Flower size

Flower measurements were made on those plant species whose pollen grains were measured for size and volume. Maximum size of flowers was established mainly from fresh flowers and photographs taken during fieldwork as well as from literature on the local flora. According to their maximum size, flowers were classified as either small (≤ 1 cm), medium (1–2 cm) or large (≥ 2 cm).

Statistical analysis

Correlation (Pearson coefficient) and linear regression analyses were used to examine the dataset. Shapiro–Wilk and Kolmogorov–Smirnov tests for normality were applied to the data set. Mean pollen volume, maximum pollen size and maximum flower size were log-transformed prior to analysis to meet the assumptions of parametric tests. To investigate whether the distribution of the pollen types by volume/size category differed in nest stores and forest, the null hypothesis was that there is no difference between the two distributions. This was tested using

a χ^2 goodness-of-fit test, at 5% significance level. The categories very small and very large had an expected frequency count of less than five. For this reason, they had to be added to small and large, respectively, as required by this test. For both statistical analyses, the program package Statgraphics® Centurion XVI (StatPoint Technologies 2010) was used.

Results

Volume range of the pollen foraged by Meliponini bees in the Dry Chaco forest

Pollen volumes varied from $565 \mu\text{m}^3$ for *Ximenesia americana* L. (Olacaceae) to $17\,450 \mu\text{m}^3$ for *Justicia* (Acanthaceae) (Figure 3). The greatest volume values calculated ($> 10\,000 \mu\text{m}^3$) were from the following families: Acanthaceae, Euphorbiaceae, Polygonaceae, Fabaceae (Caesalpinioideae), Cappariaceae, Malpighiaceae and Bignoniaceae, while the smallest ($< 2000 \mu\text{m}^3$) from the following families: Olacaceae, Asteraceae, Fabaceae (Mimosoideae), Fabaceae (Caesalpinioideae), Celastraceae, Cappariaceae, Anacardiaceae, Verbenaceae and Rhamnaceae (Figure 3).

Only two of the five volume categories were identified in the diet: small and medium (Figure 3). From the total of pollen grains measured in the nests sampled (37 pollen types), 72.97% (27 pollen types) were small (565 – $6418 \mu\text{m}^3$) and 27.03% (10 types) medium (8298 – $17\,450 \mu\text{m}^3$).

Pollen volume of the intensively foraged pollen types

The volume values of 25 out of the 32 intensively foraged pollen types ranged from $618 \mu\text{m}^3$ for *Parthenium hysterophorus* L. (Asteraceae) (small volume) to $16\,007 \mu\text{m}^3$ for *Sapium haematospermum* Müll. Arg. (Euphorbiaceae) (medium volume). When only those pollen types that were intensively foraged (>10% frequency) are considered, small pollen volumes are also highly represented (76%) (19 pollen types).

Pollen size of the foraged pollen grains

Only three of the five pollen sizes described by Wodehouse (1935) were identified in the diet of the Chaquenian Meliponini bees: small, medium and large (Figure 4). Based on the total number of pollen types measured (37), 62.16% (23 pollen types) were small (11.4–23.75 μm), 35.13% (13 types) medium (25.65–39.9 μm) and only 2.7% (one type) large (63.65 μm). When only those pollen types that were intensively foraged (>10% frequency) are considered, small pollen sizes are also highly represented (66.7%) (16 pollen types).

Flower sizes

Most of the plants (23 out of 35 species) had small-size flowers (≤ 1 cm). They were the following (in decreasing order of their pollen volume): *Sapium haematospermum* (Euphorbiaceae), *Polygonum* sp. (Polygonaceae), *Caesalpinia paraguariensis* (Fabaceae, Caesalpinioideae), *Prosopis elata* (Burkart) Burkart (Fabaceae, Mimosoideae), *Acacia praecox* (Fabaceae, Mimosoideae), *Acacia* sp., *Schinopsis balansae* (Anacardiaceae), *Achatocarpus praecox* (Achatocarpaceae), Apiaceae, *Celtis* sp. (Celtidaceae), *Castela coccoloba* (Simaroubaceae), *Schinus fasciculatus* var. *arenicola* (Anacardiaceae), *Acanthosyris falcata* (Santalaceae), *Trithrinax schizophylla* (Arecaceae), *Sideroxylon obtusifolium* (Sapotaceae), *Ziziphus mistol* (Rhamnaceae), *Phylla reptans* (Kunth) Greene (Verbenaceae), *Moya spinosa* (Celastraceae), *Maytenus vitis-idaea* (Celastraceae), *Baccharis* sp. (Asteraceae), *Pterogyne nitens* (Fabaceae, Caesalpinioideae), *Mimosa detinens* (Fabaceae, Mimosoideae) and *Parthenium hysterophorus* (Asteraceae).

Only six species had medium-size flowers (1–2 cm): *Mascagnia brevifolia* Griseb. (Malpighiaceae), *Cercidium praecox* (Ruiz et Pav. ex Hook.) Harms (Fabaceae, Caesalpinioideae), *Enterolobium contortisiliquum* (Fabaceae, Mimosoideae), *Heimia salicifolia*

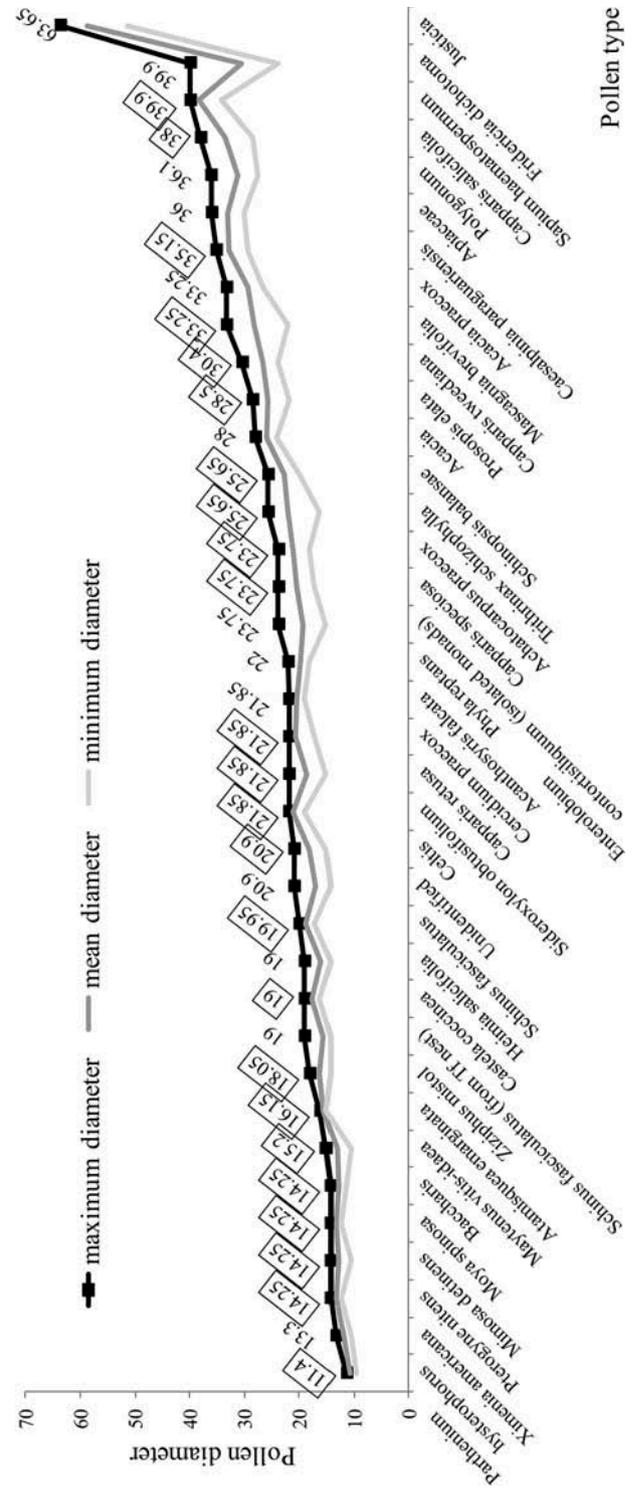


Figure 4. Maximum, minimum and mean values of the larger pollen cytoplasm diameter of 37 pollen types, in increasing order according to their maximum values. Three cytoplasmic sizes are represented: small, medium and large. Most pollen types (23) were small size and ranged from 11.4 to 23.75 μm . Values of pollen type diameters foraged intensively by at least one Meliponini species are shown within a box.

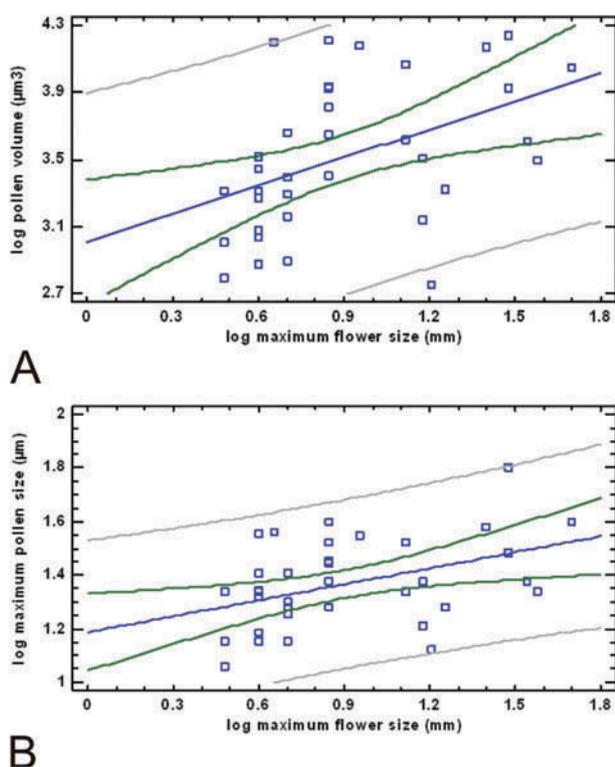


Figure 5. **A.** Correlation analysis showing the relationship between pollen volume and flower size. **B.** Correlation analysis showing the relationship between pollen size and flower size. Both analyses showed a significant moderate positive correlation (see text).

(Kunth) Link (Lythraceae), *Capparis atamisquea* (Capparidaceae) and *Ximenia americana* (Olacaceae).

Six had large-size flowers (≥ 2 cm): *Justicia* sp. (Acanthaceae), *Capparis salicifolia* Griseb. (Capparidaceae), *Fridericia dichotoma* (Jacq.) L.G. Lohmann (Bignoniaceae), *Capparis tweediana* Eichler, *Capparis speciosa* Griseb. and *Capparis retusa* Griseb.

The relationship between flower sizes and volume and size of pollen grains

A significant positive linear correlation was found between mean pollen volume and maximum flower size ($r = 0.45$, $P = 0.0055$, Figure 5A) as well as between maximum pollen size and maximum flower size ($r = 0.42$, $P = 0.0099$, Figure 5B). The maximum flower size explained moderate proportions of the variation of both the pollen grain volume (log pollen volume (μm^3) = $3.00846 + 0.55853 \cdot \log$ maximum flower size (mm); $F [1,34] = 8.79$, $P = 0.0055$) and the maximum pollen size (log maximum pollen size (μm) = $1.18686 + 0.198944 \cdot \log$ maximum flower size (mm); $F [1,34] = 7.47$, $P = 0.0099$). In both correlation analyses, most points (33) were found within

two standard deviations (mainly between one and two standard deviations) and only two points in more than two standard deviations (Figure 5).

Most of the flowers of small size had small pollen volume ranging from $618 \mu\text{m}^3$ in *Parthenium hysterophorus* to $6418 \mu\text{m}^3$ in *Acacia* (18 species). Only five pollen types corresponding to species of small-size flowers had medium volume (*Sapium haematospermum*, *Polygonum*, *Caesalpinia paraguariensis*, *Prosopis elata* and *Acacia praecox*) (Figure 3). However, most of small-size flowers (14 species) had small pollen sizes and nine flowers had medium pollen size (Figure 4) but two out of these nine species (*Acacia* sp. and *A. praecox*) presented their pollen grains clustered in polyads. For this reason, if polyad species were not taken into account, only seven species presented small-size flowers producing pollen sizes other than small.

Medium and large size flowers (12 species) had small and medium pollen volumes from 565 to $17\,450 \mu\text{m}^3$. These flowers had small, medium or large pollen sizes (seven, four and one pollen types, respectively).

Availability of pollen grains of different volumes and sizes in the xerophilous Chaco forest

Measurements of pollen grain volume (mean) ($n = 100$) and pollen grain size (larger diameter) ($n = 76$) from a total of 100 floral resources taken at random from the Dry Chaco forest were provided (Table II). The five volume and size categories were present in the available flora. For both volume and size, most pollen species belonged to small (51 and 41) and medium (32 and 22), followed by large (ten and eight), very small (five and four) and very large categories (two and one), respectively.

The χ^2 goodness-of-fit test showed significant difference between the distribution of frequencies of volume categories from nest stores versus the forest, when all pollen types ($\chi^2 = 10.07$; $P = 0.018$) as well as only the most important resources for bees ($> 10\%$) ($\chi^2 = 7.81$; $P = 0.050$) were taken into account. Bees used 27 (52.94%) out of 51 small volume species and ten (31.25%) out of 32 medium volume species available in the forest; the other three categories were not foraged. However, this test did not show a significant difference between the distribution of frequencies of size categories from nest stores and the forest for all pollen types ($\chi^2 = 3.15$; $P = 0.206$) and those represented by more than 10% ($\chi^2 = 3.22$; $P = 0.19$).

Table II. Measurements of pollen grain volume (mean) (in μm^3) and pollen grain size (larger diameter) (in μm) of 100 floral resources available in the Dry Chaco forest.

Plant family	Pollen type	Pollen grain volume	Pollen grain size	Pollen volume category	Pollen size category	Reference and sample origin ^c
Acanthaceae	<i>Justicia</i>	17 450	63.65	M	L	this article, NEST Tf ($n = 10$)
Achatocarpaceae	<i>Achatocarphus praecox</i>	4486	23.75	S	S	this article, NEST Tf ($n = 15$)
Alismataceae/	<i>Sagittaria montevidensis/</i>	5826	23.75	S	S	Vossler (2013), NEST Ga ($n = 10$)
Limnocharitaceae	<i>Hydrocleys</i>					
Amaranthaceae	<i>Alternanthera</i>	110	6.65	VS	VS	Vossler (2013), NEST Ga ($n = 4$)
Anacardiaceae	<i>Schinopsis balansae</i>	4589	25.65	S	S	this article, HERB ($n = 15$)
Anacardiaceae	<i>Schinus fasciculatus</i>	2469	19.95	S	S	this article, HERB ($n = 15$)
Anacardiaceae	<i>Schinus fasciculatus</i> (Tf)	1435	19.00	S	S	this article, NEST Tf ($n = 15$)
Apiaceae	Apiaceae	3265	36.00	S	M	this article, NEST Sj ($n = 15$)
Areaceae	<i>Trithrinax schizophylla</i>	2047	25.65	S	S	this article, NEST Tf ($n = 15$)
Asteraceae, Astereae	<i>Baccharis</i>	1009	14.25	S	S	this article, NEST Sj ($n = 15$)
Asteraceae, Astereae	<i>Baccharis/Solidago chilensis</i>	1053	15.20	S	S	Dalmazzo and Vossler unpublished, NEST Aa ($n = 15$)
Asteraceae, Astereae	<i>Solidago chilensis</i>	4123	19.87 ^a	S	S	Torres (2000), HERB ($n = 10$)
Asteraceae, Eupatoriaceae	<i>Eupatorium arnotianum</i> ^b	3976	19.62 ^a	S	S	Torres (2000), HERB ($n = 10$)
Asteraceae, Eupatoriaceae	<i>Mikania urticifolia</i>	7480	24.12 ^a	S	S	Torres (2000), HERB ($n = 10$)
Asteraceae, Helenieae	<i>Porophyllum ruderale</i>	13 440	29.37 ^a	M	M	Torres (2000), HERB ($n = 10$)
Asteraceae, Helenieae	<i>Tagetes minuta</i>	11 533	27.87 ^a	M	M	Torres (2000), HERB ($n = 10$)
Asteraceae, Heliantheae	<i>Bidens pilosa</i>	8114	24.87 ^a	S	S	Torres (2000), HERB ($n = 10$)
Asteraceae, Heliantheae	Heliantheae	1542	15.20	S	S	Vossler (2013), NEST Tf ($n = 15$)
Asteraceae, Heliantheae	Parthenium hysterophorus	618	11.40	S	S	this article, NEST Tf ($n = 15$)
Asteraceae, Heliantheae	<i>Verbesina encelioides</i>	6115	22.62 ^a	S	S	Torres (2000), HERB ($n = 10$)
Asteraceae, Inuleae	<i>Prenocaulon</i>	2577	18.05	S	S	Vossler (2013), HERB ($n = 15$)
Asteraceae, Mutisieae	<i>Holocheilus hieracioides</i>	230	7.60	VS	VS	Vossler (2013), NEST Ga ($n = 15$)
Asteraceae, Mutisieae	<i>Hyalis argentea</i> ^b	47 407	44.81 ^a	M	M	Torres (2000), HERB ($n = 10$)
Asteraceae, Vernonieae	<i>Vernonia mollissima</i> ^b	63 700	49.50 ^a	M	M	Torres (2000), HERB ($n = 10$)
Asteraceae, Vernonieae	<i>Vernonia nudiflora</i> ^b	30 739	38.75 ^a	M	M	Torres (2000), HERB ($n = 10$)
Bignoniaceae	<i>Dolichandra cynanchoides</i>	44 352		M	M	Fernández et al. (2009), HERB ($n = 10$)
Bignoniaceae	<i>Fridericia dichotoma</i>	10 954	39.90	M	M	this article, HERB ($n = 15$)
Bignoniaceae	<i>Pithecoctenium cynanchoides</i>	52 481		M	M	Fernández et al. (2009), HERB ($n = 10$)
Bignoniaceae	Tabebuia nodosa	5827	25.65	S	S	Vossler (2013), HERB ($n = 15$)
Bombacaceae	<i>Chorisia speciosa</i>	29 300		M	M	Roulston et al. (2000), HERB ($n = 5-10$)
Boraginaceae	<i>Heliotropium curassavicum</i>	2038	21.85	S	S	Vossler (2013), HERB ($n = 15$)
Bromeliaceae	<i>Dyckia</i>	3592	26.60	S	M	Vossler (2013), HERB ($n = 15$)
Cactaceae	<i>Opuntia acanthocarpa</i> ^b	427 800		L	L	Roulston et al. (2000), HERB ($n = 5-10$)
Cactaceae	<i>Opuntia arbuscula</i> ^b	414 200		L	L	Roulston et al. (2000), HERB ($n = 5-10$)
Cactaceae	<i>Opuntia phaeacantha</i> ^b	706 100		VL	VL	Roulston et al. (2000), HERB ($n = 5-10$)
Capparidaceae	Capparis atamisquea	1379	16.15	S	S	this article, NEST Tf ($n = 9$)
Capparidaceae	Capparis retusa	3133	21.85	S	S	this article, HERB ($n = 15$)
Capparidaceae	Capparis salicifolia	14 640	38.00	M	M	this article, HERB ($n = 15$)
Capparidaceae	Capparis spectiosa	4077	23.75	S	S	this article, HERB ($n = 15$)
Capparidaceae	Capparis tweediana	8344	30.40	M	M	this article, HERB ($n = 15$)
Celastraceae	Maytenus vitis-idaea	1091	15.20	S	S	this article, HERB ($n = 15$)

Celastraceae	<i>Moya spinosa</i>	1185	14.25	S	S	this article, HERB (<i>n</i> = 13)
Celtidaceae	<i>Celtis</i>	2805	20.90	S	S	this article, NEST Tf (<i>n</i> = 15)
Convolvulaceae	<i>Evolvulus sericeus</i>	29 679		M		Fernández et al. (2009), HERB (<i>n</i> = 10)
Convolvulaceae	<i>Ipomoea alba</i>	1 095 144	144.40	VL	VL	Dalmazzo and Vossler unpublished, NEST Aa (<i>n</i> = 15)
Convolvulaceae	<i>Ipomoea cairica</i>	62 476	51.30	M	L	Dalmazzo and Vossler unpublished, NEST Aa (<i>n</i> = 15)
Convolvulaceae	<i>Ipomoea hieronymi</i> ^b	231 706		L		Fernández et al. (2009), HERB (<i>n</i> = 10)
Cyperaceae	Eleocharis	470	13.30	VS	S	Vossler (2013), NEST Ga (<i>n</i> = 10)
Euphorbiaceae	Croton	32 318	45.60	M	M	Vossler (2013), NEST Ga (<i>n</i> = 15)
Euphorbiaceae	<i>Croton</i>	70 153	51.17 ^a	L	L	Silveira 1991), NEST Am (<i>n</i> = 25)
Euphorbiaceae	<i>Croton/Mamihot grahamii</i> ^b	237 593	90.25	L	L	Dalmazzo and Vossler unpublished, NEST Aa (<i>n</i> = 12)
Euphorbiaceae	Sapium haematospermum	16 007	39.90	M	M	this article, NEST Tf (<i>n</i> = 15)
Fabaceae,	Caesalpinioideae	15 133	35.15	M	M	this article, HERB (<i>n</i> = 15)
Fabaceae,	Caesalpinioideae	4168	21.85	S	S	this article, NEST Tf (<i>n</i> = 9)
Fabaceae,	Gleditsia amorphoides	5213	26.60	S	M	Vossler (2013), HERB (<i>n</i> = 15)
Fabaceae,	Pterogyne nitens	785	14.25	S	S	this article, HERB (<i>n</i> = 15)
Fabaceae,	Caesalpinioideae	21 900		M		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Fabaceae, Caesalpinioideae	<i>Senna occidentalis</i>	8298	33.25	M	M	this article, HERB (<i>n</i> = 15)
Fabaceae, Mimosoideae	<i>Acacia praecox</i>	6418	28.00	S	M	this article, NEST Sj (<i>n</i> = 15)
Fabaceae, Mimosoideae	<i>Acacia</i>	46 257	66.50	M	L	Vossler unpublished, NEST Mo (<i>n</i> = 7)
Fabaceae, Mimosoideae	<i>Albizia inundata</i>	3216	23.75	S	S	this article, HERB (<i>n</i> = 15)
Fabaceae, Mimosoideae	<i>Enterolobium</i> (isolated monad)	111 432	86.45	L	L	this article, HERB (<i>n</i> = 8)
Fabaceae, Mimosoideae	<i>Enterolobium</i> (whole polyad)	749	14.25	S	S	this article, NEST Sj (<i>n</i> = 15)
Fabaceae, Mimosoideae	Mimosa detimens	8659	28.50	M	M	this article, HERB (<i>n</i> = 15)
Fabaceae, Mimosoideae	Prosopis elata	9100		M		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Fabaceae, Papilionoideae	<i>Vicia grandiflora</i> ^b	6200		S		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Lamiaceae	<i>Salvia</i>	8266	34.20	M	M	Vossler (2013), NEST Ga (<i>n</i> = 10)
Loranthaceae	Struthanthus uraguensis	2111	19.00	S	S	this article, HERB (<i>n</i> = 15)
Lythraceae	<i>Heimia salicifolia</i>	11 595	33.25	M	M	this article, NEST Tf (<i>n</i> = 15)
Malpighiaceae	Mascagnia brevifolia	195 300		L		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Malvaceae	<i>Gossypium thurberi</i> ^b	213 151	80.75	L	L	Dalmazzo and Vossler unpublished, NEST Aa (<i>n</i> = 8)
Malvaceae	<i>Pavonia</i>					
Malvaceae	<i>Sphaeralcea ambigua</i> ^b	41 600		M		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Malvaceae	<i>Sphaeralcea cordobensis</i> ^b	23 807		M		Fernández et al. (2009), HERB (<i>n</i> = 10)
Nyctaginaceae	<i>Pisonia zapallo</i>	5566	28.50	S	M	Vossler unpublished, NEST Mo (<i>n</i> = 15)
Oleaceae	<i>Ximelia americana</i>	565	13.30	S	S	this article, NEST Tf (<i>n</i> = 7)
Passifloraceae	<i>Passiflora caerulea</i> ^b	70 997		L		Fernández et al. (2009), HERB (<i>n</i> = 10)
Plantaginaceae	<i>Scoparia</i>	46	5.70	VS	VS	Vossler (2013), NEST Ga (<i>n</i> = 6)
Polygonaceae	<i>Polygonum</i>	15 745	36.10	M	M	this article, NEST Sj (<i>n</i> = 15)

(Continued)

Table II. (Continued).

Plant family	Pollen type	Pollen grain volume	Pollen grain size	Pollen volume category	Pollen size category	Reference and sample origin ^c
Polygonaceae	<i>Ruprechtia triflora</i>	18 104	38.00	M	M	Vossler (2013), NEST Ga (<i>n</i> = 13)
Portulacaceae	<i>Portulaca</i>	248 816	96.90	L	L	Vossler (2013), NEST Ga (<i>n</i> = 14)
Ranunculaceae	<i>Clematis montevidensis</i>	2156	19.00	S	S	Vossler unpublished, HERB (<i>n</i> = 15)
Rhamnaceae	<i>Ziziphus mistol</i>	1963	18.05	S	S	this article, NEST Tf (<i>n</i> = 15)
Rubiaceae	<i>Borreria densiflora</i>	2825		S	S	Fernández et al. (2009), HERB (<i>n</i> = 10)
Santalaceae	<i>Acanthosyris falcata</i>	2070	21.85	S	S	this article, NEST Tf (<i>n</i> = 9)
Sapotaceae	<i>Sideroxylon obtusifolium</i>	2041	21.85	S	S	this article, HERB (<i>n</i> = 15)
Simaroubaceae	<i>Castela coccinea</i>	2519	19.00	S	S	this article, NEST Ga (<i>n</i> = 15)
Solanaceae	<i>Nicotiana longiflora</i>	9412		M		Fernández et al. (2009), HERB (<i>n</i> = 10)
Solanaceae	<i>Solanum</i>	1061	14.25	S	S	Vossler unpublished, NEST Mo (<i>n</i> = 15)
Solanaceae	<i>Solanum eleagnifolium</i>	10 300		M		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Solanaceae	<i>Solanum kurtzianum</i> ^b	1360		S		Fernández et al. (2009), HERB (<i>n</i> = 10)
Solanaceae	<i>Solanum lanceifolium</i> ^b	3900		S		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Typhaceae	<i>Typha latifolia</i>	6900		S		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Ulmaceae	<i>Phyllostylon rhamnoides</i>	3248	20.90	S	S	Vossler (2013), NEST Tf (<i>n</i> = 15)
Verbenaceae	<i>Glandularia laciniata</i>	18 605		M		Fernández et al. (2009), HERB (<i>n</i> = 10)
Verbenaceae	<i>Lantana grisebachii</i> ^b	11 285		M		Fernández et al. (2009), HERB (<i>n</i> = 10)
Verbenaceae	<i>Phyla reptans</i>	1861	22.00	S	S	this article, NEST Sj (<i>n</i> = 15)
Verbenaceae	<i>Verbena</i>	270	9.50	VS	VS	Vossler (2013), NEST Ga (<i>n</i> = 9)
Viscaceae	<i>Phoradendron californicum</i> ^b	6800		S		Roulston et al. (2000), HERB (<i>n</i> = 5-10)
Zygophyllaceae	<i>Buhnesia sarmientoi</i>	919	14.25	S	S	Vossler unpublished, NEST Ga (<i>n</i> = 15)
Unidentified	<i>Unidentified</i>	765	20.90	S	S	this article, NEST Sj (<i>n</i> = 15)

Note: The pollen types intensively foraged by bees are shown in bold. Categories of both volume and size include VS (very small), S (small), M (medium), L (large) and VL (very large).

^aIndicates mean values for pollen grain size.

^bCongeneric species not recorded in the study area.

^cNumber of pollen grains measured (*n*) from reference specimens deposited in herbaria (HERB) or from nest stores (NEST) of *Geotrigona argentina* (Ga), *Scaptotrigona jujuyensis* (Sj), *Tetragonisca fiebrigi* (Tf), *Melipona orbignyi* (Mo), *Augochlora amphitrinite* (Aa), *Apis mellifera* (Am).

Discussion

A wide spectrum of pollen types in colonies of three subtropical Meliponini bees

Meliponini bees carry pollen grains in their corbícula, the most specialized structure for transporting pollen among bees (Michener et al. 1978). Corbiculate bees (tribes Apini, Meliponini, Bombini and Euglossini) as well as those with scopae with sparse hairs moisten pollen grains with nectar. This enables them to increase the size of the pollen load beyond the scopal hair length and favours the collection of a wide spectrum of pollen sizes (Thorp 2000). *Leioproctus* (Colletidae) for example collects large pollen grains (80–90 µm) from *Conospermum* (Proteaceae) (Houston 1989). The fact that Meliponini bees moisten their pollen loads with nectar could account for the great variety of pollen grain volumes and sizes foraged by the three species studied in the Chaco forest. Thus, pollen from a broad spectrum of plant species of several families can be successfully foraged allowing these eusocial bees to survive in different seasons and environments.

Small bees, small flowers and small volume and size of pollen grains

Although the three Meliponini species foraged on a large number of plants, those of small volume and size pollen grains were the most intensively foraged, followed by medium grains. Large pollen grains were scarcely gathered. Although large grains supplies larger nutrient biomass (in volume) than small ones (O'Rourke & Buchmann 1991; Vossler et al. 2010), these bees concentrated their foraging on the latter.

It is known that pollen grain volume is positively correlated to the pistil length of the flowers, due to the important role of the cytoplasm nutrients in the growth of the pollen tube, essential to fertilise the ovules (Schnack & Covas 1945; Torres 2000). Plants with small pollen grains produce greater number of grains per anther than those with large grains (Subba Reddi & Reddi 1986; Tormo Molina et al. 1996), having a higher number of male gametes available to locate a flower stigma. Meliponini bees have been considered by some authors as poor pollinators because they moisten with nectar the transported pollen load, reducing the chances of losing individual pollen grains during floral visitation and of eventually making contact with a stigma (Thorp 2000). However, the high number of small grains transported per corbícula compared to large grain sizes should increase the chance of pollen being transferred to floral stigma surface during floral visits. Moreover, Meliponini bees appear to be adequate pollinators of small flowers clustered in dense

inflorescences, due to the fact that they forage slowly on a high number of flowers in the same trip.

The floral syndromes associated to the small- to medium-sized Meliponini bees include those flowers small in size, with radial symmetry and with easy access to pollen and nectar, clustered in dense inflorescences (Ramalho 1990, 2004). Although these highly generalist bees visit a broad spectrum of flower sizes (collecting a broad spectrum of pollen volumes and sizes), they prefer small flower sizes similar to their body size, being good candidates as pollinators for this kind of flowers (Ramalho 2004). From this scenario, it appears that the high abundance of small pollen grains stored in colonies of the three studied Chaquenan species of Meliponini is due to their preference for small flowers of short pistils. Wilms et al. (1997) and Ramalho (2004) suggested that the tight relationship between Meliponini and small flowers clustered in rich inflorescences is part of a diffuse co-evolutionary process and it seems to be the cause for the high abundance of mass-flowering trees in Neotropical areas. In the present study, a significant positive linear correlation between maximum flower size and both mean pollen volume and maximum pollen size was also found (Figure 5). This tight relationship between Meliponini bees and mass flowerings has been observed in tropical areas and now can also be extended to South American subtropical xerophilous Dry Chaco forest.

Availability of pollen grains of different volumes and sizes in the xerophilous Chaco forest

As it has been observed for other Neotropical environments, the eusocial Meliponini bees forage mainly on the most locally abundant plants (Ramalho et al. 1990) to supply their large colonies throughout the year. This opportunistic foraging habit would suggest that the large number of small pollen grains found in the nest samples of the present study is due to their high availability in this subtropical forest. Moreover, a study on floral visitation of six Meliponini species in this region showed that they mainly foraged on the most diverse plant families Fabaceae (subfamilies Mimosoideae and Caesalpinioideae) and Asteraceae (Vossler 2012).

These Meliponini bees foraged mainly on the trees and perennial woody shrubs from the forest, nearby to their nests. Moreover, this woody vegetation provides a higher number of flowers per individual compared to herbaceous plants from open areas and the lower stratum of the forest (grasslands, forest edges, swarms, etc.), where their preferred nesting substrates (large tree trunk) are scarce or absent. For instance, melitophilous members of Asteraceae, Malvaceae, Cactaceae,

Acanthaceae, Fabaceae (Papilionoideae), Bromeliaceae and Convolvulaceae, among others, have large to very large pollen grains and were highly available in open areas and the lower stratum of the forest but poorly represented or absent in nest pollen stores.

The comparison of volume categories between nest stores and Chaco vegetation demonstrates that foraged pollen types do not follow the abundance pattern found in the available flora. This result indicates that bees preferred certain volume categories (small and medium volumes) that are present in small flowers of short pistils. This finding is in agreement with the hypothesis of Wilms et al. (1997) and Ramalho (2004) for Neotropical habitats. However, when the five pollen size categories were compared between nest stores and the Chaco flora, preference was not found. Therefore, the abundance of small pollen types in nest samples could be interpreted as opportunism, i.e. Meliponini bees forage floral resources in proportion to their availability in the surrounding environment. This opportunism hypothesis would indicate that a high number of plant species having pollen grains of small to medium volume and size is present in the vegetation of the subtropical Dry Chaco forest. As these two results are contradictory, both hypotheses remain open.

Conclusion

In the subtropical Dry Chaco forest, the stingless bees *Tetragonisca fiebrigi*, *Scaptotrigona jujuyensis* and *Geotrigona argentina* foraged on a large number of plants, mainly those having pollen grains of small volumes and sizes, followed by medium ones. Most of the plant species (23 out of 35 species) had small-size flowers (≤ 1 cm). Most of the flowers of small size had both small pollen volume ranging from $618 \mu\text{m}^3$ in *Parthenium hysterophorus* to $6418 \mu\text{m}^3$ in *Acacia* and small pollen sizes. A significant positive linear correlation was found between mean pollen volume and maximum flower size as well as between maximum pollen size and maximum flower size.

Two hypotheses are suitable to explain why these bees mainly foraged on small pollen grains. The first hypothesis (preference) is in agreement with the findings of Wilms et al. (1997) and Ramalho (2004) for Meliponini bees in Neotropical habitats. However, the second hypothesis (opportunistic behaviour) seems to support the absence of preference for particular pollen size, indicating that these bees forage on floral resources according to their availability in the forest. The opportunism hypothesis would indicate that a high number of plant species having pollen grains of small to medium volume and size is present in the vegetation of the subtropical Dry Chaco forest.

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