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Pollen resources partitioning of stingless bees (Hymenoptera: Apidae) from the southern Atlantic forest

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ABSTRACT. Meliponinae eusocial bees are among the most important pollinators in the Neotropics and their beekeeping has been growing as both recreational and economic activity. Information on the pollen preferences and niche overlap among species coexisting in the same location is still scarce. This study focused on the use of pollen resources by three native species of Meliponinae in the Atlantic Forest, Melipona quadrifasciata quadrifasciata (Lepeletier 1836), Scapectrotrigona depilis (Moure 1942), and Tetragonisca angustula (Latreille 1811), at two different sites. The use of pollen resources was evaluated from reserves inside hives collected monthly between February 2015 and January 2016. It was also estimated the Mean Minimal Distance (Dm) traveled by the bees to obtain pollen. The bees differed regarding the diversity of pollen types, Dm, and proportion of pollen from different plant life-form. Local factor promoted differences only for Dm. M. q. quadrifasciata foraged a low diversity of pollen species and traveled greater distances with a higher proportion of trees plus palm species. Tetragonisca angustula foraged a higher diversity of pollen and flew shorter distances with a lower proportion of trees and palms. The species factor predominates over the local factor in the clustering pattern, based on pollen profile similarity.

Keywords: niche overlap; pollination; meliponiculture; Apidae.

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Introduction

Beekeeping of native stingless bees in the Neotropics began even before the arrival of C. Columbus in America (Villanueva-G., Roubik, & Colli-Ucán, 2005), with significant growth in the last decades. Indeed, it is becoming a relevant economic activity in several countries over time (Nates-Parra & Rosso-Londoño, 2013, Jaffé et al., 2015), including pollination services for crops (Del Sarto, Peroquetti, & Campos, 2005, Santos, Roselino, Hrncir, & Bego, 2009). Besides, the eusocial stingless bees comprise 2/3 of Apoidea floral visitants in wild plants of the Atlantic Forest Biome (Wilms, Fonseca, & Engels, 1996, Ramalho, 2004). On the other hand, several Meliponinae are considered endangered species – from the 24 species native from the Rio Grande do Sul state (southernmost state of Brazil), four are on the red list (Blochtein & Marques, 2003).

Pollen is the primary protein source for eusocial bees and is, therefore, essential for hive management and reproduction. Several papers about pollen resources profile by stingless bees in the Neotropics were published. Most studies cover only one species or several species with hives that do not share the same territory (Ramalho, Kleinert-Giovannini, & Fonseca, 1990). Studies on pollen resources shared among different species on the same site are scarce and mostly focused in wild populations (Wilms et al., 1996, Carvalho, Marchini, & Ros, 1999, Oliveira, Absy, & Miranda, 2009, Santos, Carvalho, Aguiar, Macêdo, & Mello, 2013). As pollinic profiles can vary depending on sites and regions (Braga et al., 2012, Novais & Absy, 2013), cross-species comparisons of pollinics become difficult. Floral resources can be a limiting factor for stingless bees rearing (Jaffé et al., 2015). Therefore, studies on partitioning resources among species can contribute to rational Meliponinae beekeeping in meliponaries (places where hives of stingless bees are kept), helping the conservation of native species, elucidating specific pollen resource needs and the effect of coexistence with other species in the same territory in rearing conditions. Studies on trophic niche overlap in meliponaries are few. Based on a study with two species of Melipona reared in a meliponary, Ferreira and Absy (2015) suggest a high pollen niche overlap in most months, indicating a limitation to the...
maintaining colonies. Regardless of their generalist potential, Meliponinae tends to rely on a few continuous sources of pollen throughout the year (Absy, Rech, & Ferreira, 2018), favoring high niche superposition.

Overlap in resources use lead to competition, and complete niche overlaps could lead to excluding all species but one (the competitive exclusion principle, Hardin, 1960). Nevertheless, niche definition depends on spatial and temporal dimensions in terms of resource location and availability over time (Palmer, Stanton, & Young, 2003). Previous research niche overlap among bees in the Neotropics has been focused mainly on the effect of feral populations of A. mellifera over indigenous bee species, suggesting a worrisome overlap among species (Wilms et al., 1996). However, the effect of competition and possible damages to native populations are controversial matters (Roubik & Wolda, 2001, Palmer et al., 2003, Paini, 2004, Freitas et al., 2009). Moreover, a low trophic niche overlap has been observed for populations of Apidae species in wild ecosystems or agricultural context (Aguiar, Santos, Martins, & Presley, 2013, Santos et al., 2013, Carvalho, Presley, & Santos, 2014, Aguiar, Caramés, França, & Melo, 2017).

Niche overlap can be prevented through different mechanisms. Preliminary evidence indicates that different species of bees differ regarding the plant life forms as a preferential source of pollen (Rodrigues et al., 2018), which in turn is related to vertical niche partitioning (Roubik, 1995). Vertical sharing of floral resources was observed for the Trigona species in a Malaysian humid forest (Nagamitsu, Momose, Inoue, & Roubik, 1999) and among the Apidae in Panama tropical forests, although most species had no vertical niche differentiation (Roubik, 1995).

In an exploratory study, the aim was to compare the trophic niche breadth of pollen for three Meliponinae species coexisting in meliponaries. The pollinic niche breadth and the niche overlap were measured at two sites in the Atlantic Forest biome. In addition, the life forms of the plants used as a source of pollen were determined, and the distance traveled to obtain the pollen was estimated. The distance of the pollen source is a critical ecological factor since there is a trade-off between the energy and nutritional value obtained and the energy applied to its obtaining (Lihoreau, Chittka, & Raine, 2011), and the hypothesis that the distance from the pollen sources differs among species was raised.

Two different meliponaries in the same ecoregion but with two different rearing conditions were included in the study. One meliponary has the coexistence of 11 Meliponinae species, while the other has only the three species included in this study (see Mat. and Methods). It was hypothesized a higher niche overlap and pollen spectra redundancy to the first site unless there are mechanisms to avoid the niche overlap altering the pollen profile, for instance, adaptive foraging (Valdovinos et al., 2016), which could reflect in the pollen diversity. Moreover, it is expected a great estimated distance traveled by the bees to obtain pollen in the first meliponary.

The similarity among the quantitative pollen profile of the bee species from the two sites was analyzed in order to verify if the local environment is a stronger determinant than the species identity. For instance, Rezende, Absy, Ferreira, Marinho, and Santos (2019) noticed a higher similarity between colonies of the same species regardless of the site locations. The biological constraint for the pollen resource choice is a piece of critical information for Meliponinae rearing in meliponaries (Absy et al., 2018).

**Material and methods**

The studied stingless bees (Hymenoptera, Apidae, Meliponinae) were Melipona quadrifasciata quadrifasciata (Lepeletier 1836), Tetragonisca angustula (Latreille 1811), and Scaptotrigona depilis (Moure 1942). Hives of all three species were kept in two different meliponaries: in the city of Barão do Coteêpe, Rio Grande do Sul State (BC, 27°56’38” S and 52°20’46” W) and in Guatambu, Santa Catarina State (GT, 27°07’50” S and 52°47’18” W), as seen in Figure 1. The first meliponary has other nine species of Meliponinae (Melipona bicolor, M. marginata, M. rufiventris, Nannotrigona testaceicornis, Scaptotrigona postica, Scaptotrigona bipunctata, Plebeia emerina, Plebeia remota and Plebeia sp.).

The samples were collected monthly from pots, collecting only pollen accumulated from before the latest data collection. Collections were carried out in February, March, April, November, December (2015), and January 2016.

Pollen preparation was modified from Louveaux, Maurizio, and Vorwohl (1978). Briefly, pollen grains were submitted to glacial acetic acid over slides for 5 minutes and after colored with toluidine blue (O’Brien, Feder, & McCully, 1964). Slides were mounted with Kaiser’s gelatin and analyzed under bright field
Stingless bees pollen reserves in meliponaries

microscopy. Each sample (bee species x month x site), counted 500 grains of pollen. Identification was aided by catalogs (Zillikens, Steiner, & Heinle, 2009, Silva et al., 2014) and other specialized literature, as well as comparison with the pollen of known plant species observed in the surroundings of the hives that were collected and mounted to be used as voucher material.

Figure 1. Atlantic Forest distribution and geographical position of the meliponaries. GT = Guatambú, BC = Barão do Cotegipe. Brazilian states: Rio Grande do Sul (RS), Santa Catarina (SC), and Paraná (PR).

Trophic niche breadth was measured with the Shannon index. The life form was classified as tree, palm, shrub, liana, and herb, according to Font Quer (1985).

The similarity of pollen flora among sites was analyzed with the Jaccard index, and among sites and bee species with the Morisita index (Horn, 1966):

\[ C_H = \frac{2 \sum_{i=1}^{S} x_i y_i}{\left( \sum_{i=1}^{S} x_i^2 + \sum_{i=1}^{S} y_i^2 \right) XY} \]

In which:
- \( x_i \) is the number of pollen grains of species \( i \) from reserves of bee species \( x \)
- \( y_i \) is the number of pollen grains of species \( i \) from reserves of bee species \( y \)
- \( X \) and \( Y \) represent the number of pollen grains counted on each sample (= 500 in this study).

A dendrogram of similarity was generated based on Morisita index. The niche overlap index (Schoener, 1968) was used to compare the species in relation to the pollen profile:

\[ NO = 1 - 0.5 \sum \left| p_{ix} - p_{iy} \right| \]

In which:
- \( p_{ix} \) is the proportion of pollen species \( i \) from bee \( x \)
- \( p_{iy} \) is the proportion of pollen species \( i \) from bee \( y \)

An estimation of the Mean Minimal Distance necessary to obtain the pollen was obtained with:

\[ D_m = \sum_{i=1}^{n} di pi \]

In which \( D_m \) is the Mean Minimal Distance (meters) needed to achieve the pollen for each bee species and month;
- \( di \) is the distance from the nearest flowering specimen of the plant species \( i \);
- \( pi \) is the proportion of the pollen species \( i \);
It should be stressed that $D_m$ is the minimal distance to be traveled for reaching the species $i$. If there were more than one available plant of the species $i$, only the distance of the nearest plant was considered. In this regard, $D_m$ has only one estimative meaning.

Statistics

The bee species were compared for monthly richness and diversity (Shannon) of pollen types and the Mean Minimal Distance ($D_m$) with a two-factor ANOVA (bee species and sites) with repeated measures (months).

The proportion of pollen among different life forms was compared with the Fisher exact test (Zar, 1999), by grouping pollen in two categories: trees + palms, and other life forms (shrubs, herbs, and lianas). Unidentified pollen species were excluded from the analysis.

The Wilcoxon Signed-Rank test was applied to compare the niche overlap values between sites.

Results

During the six months included in the sample and from reserves of all three bee species, 29 pollen species were identified in Guatambu (GT), and 27 were identified in Barão do Cotegipe (BC). It was a total of 36 different pollen types identified in both sites (20 species in common), with a Jaccard index of 0.436 between the two pollen floras.

Considering both sites, the main pollen types for *M. quadriafasciata* were *Eucalyptus grandis* H. Will. (45.9%) and *Solanum variabile* Mart. (15.6%). For *S. depilis*, *E. grandis* (34.8%) and *Syagrus romanzoffiana* (Cham.) Glassman (23.8%) predominated, and for *T. angustula*, *S. romanzoffiana* (37.6%) and *Raphanus raphanistrum* L. (26.1%) were the main pollen species.

The monthly richness of pollen types was different among bee species (two-way ANOVA, $F=10.18$, GL = 2, 20, $p = 0.0009$). *T. angustula* collected the greatest diversity of pollen, while *M. quadriafasciata* was the species with the lowest diversity (Table 1). However, local factor did not promote a significant difference ($F = 0.4957$, GL = 1, 20, $p = 0.4975$). Similarly, the monthly value of Shannon index was different among bee species (two-way ANOVA, $F=7.869$, GL = 2, 20, $p = 0.003$, Figure 2) but not among sites (two-way ANOVA, $F=0.1594$, GL = 1, 20, $p = 0.6981$). *Melipona quadriafasciata* had lower richness and Shannon values than *T. angustula* (Tukey test, $p < 0.05$). *Scaptotrigona depilis* presented an intermediary value without a significant difference to the other species (Table 1, Figure 2).

Table 1. The monthly mean richness of pollen in reserves from each bee species in both sites, Guatambu (GT) and Barão do Cotegipe (BC), Mq = *Melipona quadriafasciata*, Sd = *Scaptotrigona depilis*, Ta = *Tetragonisca angustula*. Different letters indicate significant differences between species in each site (Tukey test, $p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>GT</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mqq</td>
<td>2.50 ± 1.38 a</td>
<td>2.50 ± 1.64 a</td>
</tr>
<tr>
<td>Sd</td>
<td>4.17 ± 2.79 ab</td>
<td>4.00 ± 1.26 ab</td>
</tr>
<tr>
<td>Ta</td>
<td>4.67 ± 2.16 b</td>
<td>6.33 ± 1.03 b</td>
</tr>
</tbody>
</table>

Figure 2. Monthly Shannon index ($H'$) of pollen types from Meliponinae reserves in Guatambu (A) and Barão do Cotegipe (B) between February 2015 and January 2016. Mq = *Melipona quadriafasciata*, Sd = *Scaptotrigona depilis*, Ta = *Tetragonisca angustula*. The monthly value of the Shannon index was different among bee species (two-way ANOVA $F=7.869$, GL = 2, 20, $p = 0.003$).
In both sites, most pollen species were trees and palms (20 species = 55.6%), followed by the shrubs (eight species) and herbs (five species). The lianas were only three species. At least 63% of the pollen units for all bee species and sites were from trees and palms (Table 2). Bee species differed in the proportion of life forms used as a pollen source (Table 2). *Melipona quadrifasciata* utilized a larger proportion of trees and palms than the other species (Exact Fisher’s Test, p < 0.001).

**Table 2.** Proportion (%) of pollen for each plant lifeform according to the bee species and sites (Guatambu - GT and Barão do Cotegipe - BC). The species differed in the proportion of pollen from different life forms (grouping together: trees + palms and other life forms in other groups (shrubs, herbs, and lianas; Exact Fisher’s Test, p < 0.001).

<table>
<thead>
<tr>
<th></th>
<th>M q. q.</th>
<th>S. d.</th>
<th>T. a.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GT</td>
<td>BC</td>
<td>GT</td>
</tr>
<tr>
<td>Tree + palm</td>
<td>84.16</td>
<td>92.75</td>
<td>67.66</td>
</tr>
<tr>
<td>Shrubs</td>
<td>15.84</td>
<td>-</td>
<td>6.14</td>
</tr>
<tr>
<td>Herbs</td>
<td>-</td>
<td>7.25</td>
<td>22.86</td>
</tr>
<tr>
<td>Lianas</td>
<td>-</td>
<td>1.00</td>
<td>2.75</td>
</tr>
<tr>
<td>Unidentified</td>
<td>-</td>
<td>-</td>
<td>2.35</td>
</tr>
</tbody>
</table>

*Mq = Melipona quadrifasciata, Sd = Scaptotrigona depilis, Ta = Tetragonisca angustula*

The niche overlap (NO) between species at the same site varied between 0.022 (*T. angustula X M. quadrifasciata* in GT) and 0.465 (*S. depilis X M. quadrifasciata* in BC) (Table 3). The average values of niche overlap (NO) were 0.26 in GT and 0.39 in BC, which were not significantly different, according to the Wilcoxon Signed-Rank test (p = 0.50).

The clustering pattern generated from the Morisita index indicates that the species factor predominated over the local factor (Figure 3). Both *M. quadrifasciata* and *S. depilis* presented high similarity between conspecific hives. However, the hives of *T. angustula* exhibited low similarity between one another.

**Table 3.** Niche overlap according to the Schoener index between hives of different species in each site.

<table>
<thead>
<tr>
<th></th>
<th>Sd. GT</th>
<th>Ta. GT</th>
<th>Sd. BC</th>
<th>Ta. BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mq. GT</td>
<td>0.296</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sd. GT</td>
<td></td>
<td>0.451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mq. BC</td>
<td>0.465</td>
<td>0.262</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sd. BC</td>
<td></td>
<td></td>
<td>0.437</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.** Similarity dendrogram for bee species according to the Morisita index. *Mq = Melipona quadrifasciata, Sd = Scaptotrigona depilis, Ta = Tetragonisca angustula, BC = Barão do Cotegipe, GT = Guatambu.*

The bee species differed in $D_m$ value (two-way ANOVA, F= 7.623, Df= 2, 14, p = 0.0023); however, the distances were not significantly different between sites (two-way ANOVA, F= 7.085, Df= 1, 14, p = 0.9642). *M. quadrifasciata* collected pollen at greater distances than the other species, both in GT and BC. In turn, *T. angustula* traveled smaller distances (Table 4).

Pollen richness and $D_m$ were inversely correlated with a $P$ slightly above the level of significance (Spearman Rank Correlation = - 0.847, p = 0.0585). The local factor interfered with that correlation, that is, it was necessary a greater fly distance in BC to obtain the same pollen richness (Figure 4).
**Table 4.** Mean Minimal Distance (D<sub>m</sub>) of pollen collection for the six-sampled months on both sites (in meters).

<table>
<thead>
<tr>
<th></th>
<th>M. quadrifasciata</th>
<th>S. depilis</th>
<th>T. angustula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guatambu</td>
<td>121.4 ± 45.2</td>
<td>86.7 ± 39.6</td>
<td>63.3 ± 31.9</td>
</tr>
<tr>
<td>Barão do Cotegipe</td>
<td>229.0 ± 81.5</td>
<td>165.2 ± 92.3</td>
<td>74.4 ± 58.0</td>
</tr>
</tbody>
</table>

**Figure 4.** Relationship between monthly richness of pollen types (R) and the Mean Minimal Distance (D<sub>m</sub>). Spearman rank correlation = - 0.847, p = 0.0583. The open and closed figures represent Guatambu and Barão do Cotegipe, respectively.

**Discussion**

*Melipona quadriafasciata* presented the highest level of pollen foraging specialization, not surpassing five pollen species each month, and foraging only one species on both sites in March and April 2015. Moreover, *M. quadrifasciata* had the highest proportion of pollen from tree species, while other life forms account for less than 16% and 8% of pollen in the sites GT and BC, respectively. A low diversity of floral resources for *M. quadrifasciata* was previously observed by Wilms et al. (1996), and a preference for larger species was reported by Ramalho (2004) and Rodrigues et al. (2018). The need for large plant species could be a limiting factor for *M. quadrifasciata*, making its adaptation in meliponaries dependent on specific floral resources. Thus, forest remnants or plantation of suitable tree species could be decisive to the beekeeping of *M. quadrifasciata* without artificial feeding.

At the other end, *T. angustula* presented the higher diversity of pollen types on both sites, indicating a more generalist behavior. Moreover, the proportion of pollen from non-tree species reaches 29-30% for *T. angustula*, which could contribute to avoiding niche overlap. These findings contrast with the observations by Ramalho (2004), who reported that less than 15% of *T. angustula* workers were observed at flowers under 7.0 meters tall in Atlantic Forest remnants. On the other hand, in the agriculture landscape, the preference for herbs and shrubs (including ruderal and cultivated species) can increase the risks of agrochemicals contamination in honey (Rodrigues et al., 2018).

The low diversity of pollen types for *M. quadrifasciata* was associated with greater distances to obtain pollen (D<sub>m</sub>), which can reinforce the hypothesis of higher selectivity. It should be stressed that D<sub>m</sub> is just an estimation of the minimal flying distance, meaning that distances could be farther (although not shorter). Nevertheless, this estimate allows comparisons among species that could be easily calculated with data on local vegetation.

*Melipona quadriafasciata* and *S. depilis* bees presented high similarity between conspecific hives in the two different sites. In other words, the local factor was less important than the species identity in the selection of the pollen species, reinforcing the importance of the specific pollen resources locally available to better results in the meliponaries (Ferreira & Absy, 2015). On the other hand, *T. angustula* showed a different behavior, with a low similarity between sites and a higher diversity of pollen types.

The data indicate that *T. angustula* combined a wider diversity of pollen sources with short foraging distances, adjusting its pollen profile according to local availability. This data corroborates with the generalist profile attributed to this species (Carvalho et al., 1999), which can contribute to its remarkable ease in beekeeping (Braga et al., 2012, Silva, Ramalho, & Monteiro, 2013).
Trophic niche breadth, diversity, and overlap

Morisita values among species pairs presented a mean of 0.40 for the GT site (0.05-0.65) and of 0.55 for the BC site (0.56-0.79), which can be considered relatively low, considering that Carvalho et al. (1999) obtained a value of 0.78 between T. angustula and Nannotrigona testaceicornis for the same index.

Niche overlap according to the Schoener index varied under 0.6, even considering pollen foraging spectra of the entire studied period, which can be considered low according to Ferreira and Absy (2015). On the other hand, NO values based on flower visitation in the Caatinga Biome varied between 0.09 and 0.61 (mean= 0.33) among Meliponinae species (Santos et al., 2013). Therefore, our values between 0.02 and 0.46 (mean= 0.32) are in the same range. However, the lack of reference values in wild or meliponary communities from Southern Brazil limits the comparative analysis of trophic niche partitioning.

Higher values of $D_n$ at BC can be explained by the larger number of bee species being kept together. Hive density in the wild is lower than 3.0 ha$^{-1}$, though the available hollow trunks for nesting are not occupied entirely, which indicates that food is the most limiting resource (Eltz, Brühl, Van der Kaars, & Linsenmair, 2002, Silva et al., 2013). Thus, competition in meliponaries tends to be elevated to the extent that the number of colonies and species is increased.

Conclusion

The data reaffirmed differences among species concerning the diversity of pollen foraging. Additionally, differences among bee species in the proportion of pollen from different plant life forms and in the distance traveled to the pollen sources were detected. Except for T. angustula, with great adaptive foraging, the other two species maintain their pollen species preferences regardless of the local. The data corroborate that the higher popularity of T. angustula among beekeepers is related to its generalist profile, adjusting pollen collection to the flora available in a short-range around the hives. On the other hand, M. quadrifasciata mainly requires pollen from trees and palms and is highly selective for pollen species. The local factor did not change niche overlap (contrary to what was supposed), but rather the distance from pollen sources.

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References


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