Pollen Resources Used by Two Species of Stingless Bees (Meliponini) in a Tropical Dry Forest of Southern Ecuador

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Abstract

Pollinators are crucial for ecosystem functionality; however, little is known about the plant species used by some of these, such as stingless bees. In this study, for the first time, pollen resources used by Melipona mimetica Cockerell (Hymenoptera: Apidae: Meliponini) and Scaptotrigona sp. Moure (Hymenoptera: Apidae: Meliponini) were identified through analysis of corbicular pollen found on worker bees in a dry forest in southern Ecuador. In total, 68 pollen types were identified belonging to 31 botanical families. The most represented plant families were Fabaceae (16%), Malvaceae (7%), and Boraginaceae (7%). Both stingless bee species exhibited a polylectic behavior, with an average of 16 pollen types collected by individual bees. Differences in abundances of pollen types collected by each species indicated distinct uses for these two bee species.

Key words: stingless bee, corbicular pollen, Melipona, Scaptotrigona, dry forest

Pollinators play a critical functional role in most terrestrial ecosystems because plant persistence and regeneration depend on their activity (Klein et al. 2007, Aby et al. 2018). Additionally, they provide a key ecosystem service not only through the pollination of many crops but also through the direct resources they offer local human communities such as honey (Pasupuleti et al. 2017). Among pollinators, bees are particularly important in the reproduction of crops and most wild plants (Klein et al. 2007, Garibaldi et al. 2013). In parallel, plants are essential for bee growth and reproduction because, in all their life stages, bees are nutritionally dependent on floral resources, mainly nectar and pollen (Ogilvie and Forrest 2017, Aby et al. 2018). Unfortunately, pollinator decline is invoked as one of the most pervasive problems worldwide (Potts et al. 2010). Consequently, an understanding of bees’ performance and resource preferences in different ecosystems will permit the development of conservation actions to mend the decline in pollinators caused by human activities (Kleijn et al. 2008, Potts et al. 2010, Roubik and Patiño 2018).

Stingless bees comprise a diverse group of eusocial bees, especially diversified in the tropics (Slaa et al. 2006). They are considered one of the most important pollinators (Quezada-Euán 2018) and, also, the most relevant as ecosystem service providers in several tropical ecosystems. They are considered polylectic (i.e., generalists) because of their ability to collect pollen and nectar from an array of nonrelated plants (Eltz et al. 2001, Biesmeijer et al. 2005). Consequently, stingless bees constitute an important component in the complex pollinator networks of most tropical forest ecosystems (Schleuning et al. 2012). To know how these bees forage in tropical forests is a priority to guarantee their conservation and the services these insects provide (Murray et al. 2009).

Ecuador is considered one of the most diverse stingless bee hotspots with ca. 130 species representing 25% of the known species worldwide (Roubik 2018, Vit et al. 2018). An example of this extraordinary diversity was documented by Roubik (2018) in a 50 ha plot in Yasuni Biosphere Reserve, where 100 stingless bee species were identified. In addition, in the seasonal forests of southern Ecuador, 89 species have been recorded (Ramírez-Romero et al. 2013), some of which are traditionally managed in rural–urban areas and critical for the local economies (Martínez-Fortín et al. 2018). Surprisingly, information on the foraging behavior of stingless bees is almost entirely lacking for some Neotropical countries, including Ecuador.

Therefore, our research objective is to identify the pollen sources used by Scaptotrigona sp. Moure (Hymenoptera: Apidae: Meliponini) ‘catana’ and the Melipona mimetica Cockerell (Hymenoptera: Apidae: Meliponini) ‘bermejo’, which are two of the most important bees managed for honey production in seasonal dry forests in southern Ecuador (Vit et al. 2018). These forests are one of the most threatened ecosystem in Ecuador and are suffering a dramatic decline with unknown deforestation rates (Linares-Palomino et al. 2011, Tapia-Armijos et al. 2015).
Materials and Methods

The present study was conducted in the La manga-Garza Real area (85.1987 S; 72.712977 W) located in the municipality of Zapotillo, Loja Province, in the Southwestern of Ecuador. The regional climate is tropical semiarid, with an average annual temperature of 24°C and an average annual rainfall of 500 mm with a dry season between May and December and a rainy season between January and April (Maldonado 2002). This area is characterized by dry seasonal forests dominated by tree species such as Guayacán (Handroanthus chrysanth), Ceiba (Ceiba trichistandra), and Pretino (Cavanillesia plataniifolia), which are interspersed with lowland scrub formations at elevations ranging between 525 and 547 m.a.s.l. (Cueva and Chalán 2010).

Sampling was carried out in the months of June, August, October, and November 2015 and May 2016, covering both dry and rainy seasons. We selected 3 meliponaries, spaces for the breeding of stingless bees for honey production, separated 500 m from each other, for evaluating the foraging behavior of both species. Each meliponary contained a colony of each stingless bee species. Pollen foragers returning to the hive were captured at the nest entrances using a hand-net between 8:30 and 14:00 h. From each colony, samples were collected from two pollen sacs of foragers bees, and each sample was individually placed in a 2-ml Eppendorf tube, duly labeled, and brought back to the laboratory. During each month, pollen was collected from 30 foragers for each colony and bee species.

Due to the lack of a pollen library for the plant species in our study region, pollen characterization of the vegetation was necessary. Floral resources identification in the surrounding habitat was done through a radial track of 2 km around the colonies; this distance is an approximate foraging range of some stingless bee species (Kuhn-Neto et al. 2009). For each plant species with floral resources, blossoms and flowers were taken for pollen characterization. We used these samples to create a pollen reference collection for our study.

In the laboratory, corbicular pollen samples from each individual were diluted in alcohol and mounted on three microscope slides. Once the alcohol evaporated, we set the pollen using 2% solution; the center of each slide was stained with fuchsin. The glycerogelatin solution was prepared according to Wodehouse (1935), diluting 7 g of gelatin, 50 ml of glycerin, and 1 g phenol in 42 ml of distilled water. The classification of pollen morphotypes was done using a Zeiss—Axiostar Plus optical microscope with a 40x objective lens. The number of grains of each morphotype was recorded for each slide, and relative abundance of each representative plant taxon found in the pollen sample was quantified for the three slides. The acetylation method was not used; therefore, some morphological characteristics of the pollen were not described. Taxonomic identification of pollen morphotypes found on stingless bees to the level of family, genus, or species was done by comparing them to the hand-collected pollen of plants in the area and, in some cases, with information about the characteristics of the family. Additionally, we obtained the mean and SD of pollen morphotypes visited by individual bees. We calculated the number of pollen types collected by an individual bee by summing the pollen types found on the three slides examined per bee.

Results

In total, 251 individuals of Melipona mimetica and 244 individuals of Scaptotrigona sp. that presented pollen loads were analyzed. In total, we identified 68 pollen morphotypes belonging to 31 families, 38 genera, 36 species, and 10 undetermined types (Table 1, Supp Plates 1–5 [online only]).

The occurrence of pollen morphotypes (for pooled samples) were almost identical for these two species: 66 for M. mimetica and 67 for Scaptotrigona sp. The botanical families that presented more pollen morphotypes were Fabaceae (11), Malvaceae and Boraginaceae (5), and Lamiales (3; Table 1). The two bee species collected similar pollen morphotypes although in different relative abundances (Fig. 1). For M. mimetica, the most abundant pollen morphotypes (>5%) were from Corchorus sp. (Tiliaceae) with 14% of total corbicular pollen samples collected in the whole sampling period, 13% Cunoniaceae type 1, 8% Polygonaceae type 1, 7% Byttneria flexuosa (Sclerocaulaceae), and 5.2% Cochlospermum vitifolium (Bixaceae) (Table 1, Fig. 1). For Scaptotrigona sp., the most representative species were as follows: Polygonaceae type 1 with 20%, Corchorus sp. with 13%, Byttneria flexuosa with 10%, Muntingiaceae type 1 species with 10%, and Crotolaria sp. (Fabaceae) with 7% (Table 1, Fig. 1).

Additionally, we found a polylectic behavior for both bee species, with a mean of 16.51 (±4.89 SD) pollen morphotypes collected by individuals of M. mimetica and 15.95 (±5.96 SD) for Scaptotrigona sp. Although both bee species collected the same number of pollen morphotypes, M. mimetica bees collected more than 20% of the pollen from a mean of 1.42 (±0.56 SD) morphotypes, in contrast to 1.28 (±0.62 SD) morphotypes for Scaptotrigona sp.

Discussion

In this study, the plant families Fabaceae, Malvaceae, and Boraginaceae represented the highest number of pollen morphotypes. These findings agree with other studies that have reported similar results for eusocial bees, such as those of the Meliponini tribe and Apis mellifera L. (Apini) both in genuine drylands and tropical regions (Dórea et al. 2010, Faria et al. 2012, de Novaïs et al. 2015). The Fabaceae family is identified in most previous studies as the most important pollen source both in terms of the number of pollen morphotypes and their relative abundances (e.g., Ramalho et al. 1990, Faria et al. 2012, de Novaïs et al. 2015). In the present study, these results are not unexpected as the three plant families are also the richest ones in the aboveground vegetation of our study area (Cueva-Ortiz et al. 2019). In contrast, pollen morphotypes of the other families, such as Tiliaceae, Cunoniaceae, Polygonaceae, Muntingiaceae, Sterculiaceae, which were identified as primary foraging resources for both M. mimetica and Scaptotrigona sp. in the present study, have not been previously reported for other stingless bee species. For example, Barros et al. (2013) and Ferreira and Absy (2017) in the Brazilian Amazon identified Melastomataceae and Solanaceae as the most representative resources for Melipona interrupta and Melipona fasciculata, respectively. Likewise, Rech and Absy (2011) determined that Fabaceae, Bignoniaceae, and Lamiaceae were important plant families for Scaptotrigona sp. However, most studies took place in wet regions and further research on stingless bees’ pollen resources in dry ecosystems is necessary to confirm the generality of the patterns reported in the present study.

Both stingless bees, M. mimetica and Scaptotrigona sp., exhibited a polylectic behavior with a total of ca. 65 pollen morphotypes collected for each bee species and with individual bees collecting on average 16 different pollen morphotypes. This polylectic behavior has been evidenced in other stingless bee species, with some species collecting 100 different pollen morphotypes (e.g., Heithaus 1979, Wilms et al. 1996). However, other bee species within the Melipona genus do not collect such a high number of pollen morphotypes. For example, Melipona subnitida collected only 14 different morphotypes in a dry
| Family          | Species/pollen type         | Melipona mimetica | Scaptotrigona sp. | Plant type | Plate |
|-----------------|----------------------------|-------------------|-------------------|------------|-------|
| Apocynaceae     | Nerium oleander            | 0.34              | 0.69              | Tree       | 1-p   |
| Areaceae        | type 1                     | 0.55              | 0.24              | 2-f        |
| Asteraceae      | Parthenium hysterophorus   | 3.32              | 2.57              | Herb       | 1-r   |
|                 | Juncus paniculatus         | 1.32              | 0.53              | Herb       | 1-h   |
| Bidens pilosa   |                            | 0                 | 0.001             | Herb       | 5-i   |
| Bixaee          | Cochlospermum vitifolium   | 5.12              | 1.81              | Tree       | 2-d   |
| Boraginaceae    | Heliotropium angiospernum  | 0.68              | 0.54              | Herb       | 1-g   |
|                 | Cordia sp.1                | 0.44              | 0.57              | Shrub      | 2-p   |
|                 | Cordia longa               | 0.05              | 0.02              | Shrub      | 2-g   |
|                 | Heliotropium sp.1          | 3.68              | 2.71              | Herb       | 2-a   |
|                 | type 1                     | 0.14              | 0.42              | 3-d        |
| Cactaceae       | Armatocereus cartwrightianus | 0.66           | 0.41              | Succulent  | 2-h   |
|                 | Opuntia megasperma         | 0.12              | 0.02              | Succulent  | 5-e   |
| Convolvulaceae  | Ipomoea sp.1               | 0.19              | 1.52              | Shrub      | 4-h   |
| Cunoniaceae     | type 1                     | 12.58             | 0.77              | 5-b        |
| Eriaceae        | type 1                     | 1.39              | 0.78              | 2-o        |
| Euphorbaceae    | Croton scouleri            | 0.43              | 0.26              | Shrub      | 3-f   |
| Fabaceae        | Caesalpinea glabra         | 4.13              | 1.2               | Tree       | 5-c   |
|                 | Patthelobium sp.1          | 2.38              | 1.55              | Tree       | 1-k   |
|                 | Crotalaria sp.1            | 1.55              | 7.56              | Herb       | 1-s   |
|                 | Canavus cajan              | 0.99              | 0.54              | Shrub      | 2-j   |
|                 | Cecidium praecox           | 0.95              | 0.97              | Tree       | 3-e   |
|                 | Senna incarnata            | 0.57              | 0.81              | Shrub      | 1-i   |
|                 | Leucaena leucocephala      | 0.25              | 0.32              | Tree       | 2-k   |
|                 | Prosopis julifora          | 0.12              | 0.04              | Tree       | 5-f   |
|                 | Erythrina velutina         | 0.05              | 0.16              | Tree       | 1-m   |
|                 | Acacia riparia             | 0.007             | 0.01              | Tree/shrub | 3-a   |
|                 | Bauhinia aculeata          | 0.002             | 0                 | Herb       | 4-e   |
| Lamiaceae       | Clerodendrum molle         | 4.6               | 4.44              | Shrub      | 1-b   |
|                 | type 1                     | 0.65              | 0.29              | Shrub      | 1-n   |
|                 | type 2                     | 0.03              | 0.02              | Shrub      | 4-f   |
| Malvaceae       | Eriotheca ruizi            | 0.43              | 0.63              | Tree       | 1-c   |
|                 | Ceiba insignis             | 0.06              | 0.04              | Tree       | 1-d   |
|                 | Ceiba trichistandra        | 0.06              | 0.03              | Tree       | 2-q   |
|                 | Ochroma pyramidale         | 0.03              | 0.01              | Tree       | 4-c   |
|                 | Biquetia spicata           | 0.02              | 0.01              | Herb       | 4-b   |
| Melastomataceae | Miconia gleasoniana        | 0.7               | 1.64              | Shrub      | 1-t   |
| Mimosaceae      | Inga cornascans            | 1.16              | 0.4               | Tree       | 1-l   |
|                 | Inga sp.                   | 0.007             | 0.01              | Tree       | 3-b   |
| Muntingiaceae   | type 1                     | 3.42              | 10.24             | Shrub      | 1-q   |
| Myrtaceae       | Psidium sp.1               | 0.08              | 0.03              | Tree       | 4-g   |
|                 | type 1                     | 0                 | 0.01              | 5-l        |
| Nyctaginaceae   | Bougainvillea sp.1         | 0.22              | 0.13              | Shrub      | 5-a   |
| Oleaceae        | type 1                     | 0.17              | 0.12              | Shrub      | 2-r   |
| Plumbaginaceae  | Plumbago scandens          | 0.74              | 1.39              | Herb       | 2-t   |
| Polygonaceae    | type 1                     | 7.97              | 19.77             | 1-j        |
|                 | type 2                     | 1.04              | 0.09              | 2-n        |
| Proteaceae      | type 1                     | 2.21              | 1.02              | Shrub      | 2-b   |
In a similar fashion, *Melipona interrupta* (Ferreira and Absy 2017), *Melipona fasciculata* (Barros et al. 2013), and *Melipona capixaba* (Luz et al. 2011) from wetter tropical forests foraged on approximately 30 different pollen morphotypes, which is still far below what was observed in our species of *Melipona*.

In Brazil, *Scaptotrigona fulvicutis* in a semideciduous forest (Marques-Souza et al. 2007) and *Scaptotrigona aff. depilis* in central Amazonian (Faria et al. 2012) also showed a very high number of resource plants, collecting 85 and 97 different pollen morphotypes, which is more pollen types than that reported for our *Scaptotrigona* species.

In conclusion, this study provides basic information on the plant resources visited by these two stingless bee species in southern Ecuador. Because of their endangered status and local economic importance, knowledge of the pollen resources used by these two bee species could help beekeepers better manage them and develop preservation programs, e.g., concentrating efforts to recover the vegetation in deforested areas using plant species commonly visited by these bees (Luz et al. 2011).

Supplementary Data
Supplementary data are available at *Journal of Insect Science* online.

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