Hindawi Publishing Corporation
Psyche
Volume 2015, Article ID 659538, 7 pages
http://dx.doi.org/10.1155/2015/659538

Research Article

Broad Protein Spectrum in Stored Pollen of Three Stingless Bees from the Chaco Dry Forest in South America (Hymenoptera, Apidae, Meliponini) and Its Ecological Implications

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Received 26 September 2015; Accepted 2 November 2015

Academic Editor: David Roubik

Protein content of pollen stored by three meliponine species was variable from 9.78% (less than half the value considered as optimal to brood development in *Apis mellifera*) in type *Capparis tweediana-C. speciosa* to more than 26% in type *Maytenus vitis-idaea* and some *Prosopis* samples. This pollen of low protein value was occasionally foraged (only six out of 75 masses analyzed of *G. argentina*, but none in 86 masses of *T. fiebrigi* or in ten of *M. orbignyi*). However, it is likely that amino acid deficiencies of certain pollens are compensated by randomly foraging on a broad spectrum of pollen plants. The large amounts of pollen stored in their nests might also be important in compensating these deficiencies. The only sample studied for *M. orbignyi* showed a protein value greater than the one required for *A. mellifera* and was dominated by types *Acacia praecox* and *Prosopis*. As this species also prefers *Solanum* and other protein-rich pollen, more samples would need to be analyzed to establish whether protein requirements are high for this *Melipona* species. Pollen showing the highest protein content (>26%) belonged to highly nectariferous plants well represented in meliponine and *Apis* honey such as *Prosopis*, *Maytenus*, and *Ziziphus*.

1. Introduction

The stingless bees (Apidae: Meliponini) are eusocial and they build perennial nests with hundreds to thousands of individuals and high reproductive rate [1, 2]. To maintain their colonies, high amounts of pollen and nectar are foraged from flowers, the former stored as pollen masses and the latter as honey in pots made of cerumen. Pollen pots are located mainly surrounding the brood area where young individuals are growing [3]. Pollen stored in nests is chemically and biochemically different from fresh pollen from anthers or pollen loads from the same plant species due to regurgitated liquids incorporated during packing [4–7]. Mutualistic bacteria were found in stored pollen and honey of nine *Apis* species, *Melipona fasciata panamica* Cockerell, *Melipona beecheii* Bennett, *Meliponula bocandei* (Spinola), and *Trigona* producing enzymes that may facilitate storage and/or digestion by bees and secrete antibiotics and fatty acids to inhibit microorganisms that cause spoilage of stored food [8, 9]. During periods of general food shortage, *Melipona* species show conservative trends eating young larvae and provisions and drastically decreasing brood production [10]. Although exceptional protein resources such as soybean bran in *Geotrigona mombuca* (Smith) (sub *G. inusitata* in [11]) and flesh of dead animals in many *Trigona*, *Partamona*, *Oxytrigona*, *Cephalotrigona*, and others [12] can be facultatively gathered, pollen is the main protein source in bees. Obligate necrophagy (dead animal flesh is the only protein source) occurs only in *Trigona crassipes* (Fabricius), *T. hypogea* Silvestri, and *T. necrophaga* Camargo & Roubik [11, 13–15]. For *Apis mellifera*, the higher the crude protein percentage the lesser amount of pollen required to sustain production [16]. Ample protein content promotes a high birth rate and long-lived bees, 20% being the minimum protein

amount required by colonies for optimum production [17]. A well-nourished bee colony is a guarantee for good level of honey flow and breeding [16].

It is important to know pollen protein intake of meliponine bees for appropriate colony rearing (meliponiculture), as it would be useful to determine supplemental feeding needed in periods of flowering shortage. Stingless bees are important for pollination of wild and crop plants [18] and for bee-products production (honey, pollen, resin, and cerumen) [19]. The aims of the present study were to determine protein content of pollen stored in nests of three meliponine bee species (*Tetragonisca fiebrigi* (Schwarz), *Melipona orbignyi* (Guérin), and *Geotrigona argentina* Camargo & Moure) from the South American Chaco dry forest. These are three out of the seven species recorded for this area [20, 21] being important mainly for rural population for their honey, pollen masses, larvae, and cerumen [22].

2. Materials and Methods

2.1. Sampling and Study Area. Pollen mass from cerumen pots was obtained from nests of *Tetragonisca fiebrigi* (Schwarz), *Melipona orbignyi* (Guérin), and *Geotrigona argentina* Camargo & Moure from xeric forests in the Chaco region of Northern Argentina (Figure 1). Samples for protein analysis were taken from nest 7 of *T. fiebrigi*, nest 11 of *M. orbignyi*, and nests 2 and 4 of *G. argentina* from El Sauzalito (24°24′S, 61°40′W), from nest 1 of *G. argentina* from El Espinillo (25° 24′S, 60° 27W), from nest 5 of *T. fiebrigi* from Miraflores (25°29′S, 61°01′W), and from nest 12 of *T. fiebrigi* from Villa Río Bermejito (25°37′S, 60°15′W). This Chaco dry forest is characterized by the dominance of "palo santo" (*Bulnesia sarmientoi* Lorentz ex Griseb., Zygophyllaceae) and "quebracho blanco" trees (*Aspidosperma quebracho-blanco* Schltdl., Apocynaceae), or by "quebracho colorado santiagueño" (*Schinopsis lorentzii* Engl., Anacardiaceae), "quebracho colorado chaqueño" (*Schinopsis balansae* Engl.), and "quebracho blanco" [23, 24]. Other woody elements well represented in this xerophilous flora are "mistol" (*Ziziphus mistol* Griseb., Rhamnaceae), "molle" or "guaraniná" (*Sideroxylon obtusifolium* (Roem. & Schult.) T. D. Penn., Sapotaceae), several species of *Prosopis* ("algarrobo blanco," "algarrobo negro," "vinal," "vinalillo," "carandá," and "guachín") (Fabaceae, Mimosoideae), "guayacán" (*Caesalpinia paraguariensis* (D. Parodi) Burkart, Fabaceae, Caesalpinioideae), "tipa colorada" or "palo coca" (*Pterogyne nitens* Tul., Fabaceae, Caesalpinioideae), "palo cruz" (*Tabebuia nodosa* (Griseb.) Griseb., Bignoniaceae), "quebrachillo" (*Aspidosperma triternatum* Rojas Acosta, Apocynaceae), "palo borracho" or "yuchán" (*Ceiba chodatii* (Hassl.) Ravenna, Bombacaceae), "palma de monte" (*Trithrinax schizophylla* Drude, Arecaceae), "meloncillo" (*Castela coccinea* Griseb., Simaroubaceae), "palo tinta" (*Achatocarpus praecox* Griseb., Achatocarpaceae), "tala" (*Celtis* spp., Celtidaceae), "duraznillo" (*Salta triflora (Griseb.) Adr. Sánchez*, Polygonaceae), "pata" (*Ximenia americana* L., Olacaceae), "molle" (*Schinus fasciculatus* (Griseb.) I. M. Johnst. var. *arenicola* (Hauman) F. A. Barkley, Anacardiaceae), "chañar" (*Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart, Fabaceae, Papilionoideae), "sal de indio" or "sal de monte" (*Maytenus vitis-idaea* Griseb., Celastraceae), "paloma yuyo" (*Moya spinosa* Griseb., Celastraceae), "teatín" (*Mimosa detinens* Benth., Fabaceae, Mimosoideae), "cardón" (*Stetsonia coryne* (Salm-Dyck) Britton & Rose, Cactaceae), "ucle" (*Cereus forbesii* Otto ex C. F. Först., Cactaceae), two species of *Bougainvillaea* ("rama overa") (Nyctaginaceae), several species of *Acacia* ("tusca," "garabato," and "churqui") (Fabaceae, Mimosoideae), *Capparis* ("atamisqui," "sacha membrillo," "sacha sandia," "sacha poroto," and "bola verde"), and "cardo" or "chaguar" (several Bromeliaceae genera), among others.

2.2. Pollen Analysis of Pollen Masses and Plant and Bee References. Pollen masses were dissolved in distilled water at 80–90°C and stirred with a magnetic stirrer for 10–15 min. A representative mixture of 5–10 mL was obtained and centrifuged at 472 ×g for 5 min. Processing included acetolysis [25]. Under a Nikon Eclipse E200 light microscope, a total of 300–500 pollen grains per slide were counted. Pollen grain identification was carried out comparing nest pollen slides with those present in the reference pollen collection. The reference slides consist of a total of 190 plant species and are deposited in PAL-CICYTTP pollen collection of Diamante, Entre Ríos, Argentina. It was made from flower buds of plant species collected in various localities from Chaco Province of Argentina. These plant specimens were pressed, dried, and identified by the author and deposited in the Herbarium of the Museo de La Plata (LP), the Herbarium of Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (BA), Buenos Aires, and the Herbarium Lorentz (DTE) of Diamante, Entre Ríos, Argentina. Plant nomenclature follows [26]. Bee specimens were collected from nests, identified by Arturo Roig-Alsina, and deposited in the Entomology Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina.

2.3. Determination of Protein Content. To quantify protein of pollen resources foraged by bees, certain pollen masses were selected according to their pollen type composition. Six pollen mass samples were taken from nests 5, 7, and 12 of *Tetragonisca fiebrigi*, one from nest 11 of *Melipona orbignyi*, and eight from nests 1, 2, and 4 of *Geotrigona argentina* (Table 1). For nitrogen content determination, 50 mg of pollen [27] was analyzed by the micro-Kjeldahl method [28] and crude protein was estimated using the factor 6.25 [29]. Pollen nitrogen content was analyzed in LANAIS N15 (National Laboratory of Research and Services UNS-CONICET), Departamento de Agronomía, Universidad Nacional del Sur, Bahía Blanca, Argentina.

3. Results

3.1. Protein Content of Pollen Stored in Meliponine Nests. Nitrogen values from pollen grains stored in pollen pots of the three meliponine species studied varied from 1.56 to 4.86%, which is equivalent to 9.78 to 30.41% of crude

Table 1: Main pollen types (>10% representation) present in pollen mass samples of *Tetragonisca fiebrigi* (*Tf*), *Melipona orbignyi* (*Mo*), and *Geotrigona argentina* (*Ga*) and their nitrogen and protein percentages.

Bee species	Sample	Nest and pollen mass	Main pollen types	Nitrogen (%)	Crude protein (%)
Tf	1	5 B	Type *Schinopsis* (59%) + *Trithrinax schizophylla* (40.5%)	3.055	19.09
	2	5 C	*Trithrinax schizophylla* (41%) + *Parthenium hysterophorus* (30%) + type *Schinopsis* (28%)	2.854	17.84
	3	5 E	*Trithrinax schizophylla* (98%)	2.898	18.11
	4	7 A	*Sideroxylon obtusifolium* (24%) + *Prosopis* (18%) + type *Maytenus vitis-idaea* (16%) + *Tabebuia* (13%)	3.126	19.54
	5	7 F	*Capparis speciosa* (42%) + *Mascagnia brevifolia* (34%) + Heliantheae (10%)	3.648	22.80
	6	12 C	*Ziziphus mistol* (60%) + *Prosopis* (30%)	3.542	22.14
Mo	7	11	Type *Acacia praecox* (53%) + *Prosopis* (38%)	3.881	24.26
Ga	8	4 58	Type *Capparis tweediana-C. speciosa* (100%)	1.56	9.78
	9	1 24	*Castela coccinea* (100%)	3.26	20.36
	10	2 31	*Prosopis* (100%)	3.34	20.87
	11	1 11	*Prosopis* (91%)	4.86	30.41
	12	2 36	*Prosopis* (89.5%)	4.32	27.03
	13	2 33	Type *Maytenus vitis-idaea* (100%)	4.23	26.87
	14	2 42	Type *Maytenus vitis-idaea* (96%)	4.24	26.51
	15	1 16	Type *Croton* (30%) + type *Sagittaria* (18%) + *Eleocharis* (12%) + *Castela coccinea* (11%)	2.65	16.59

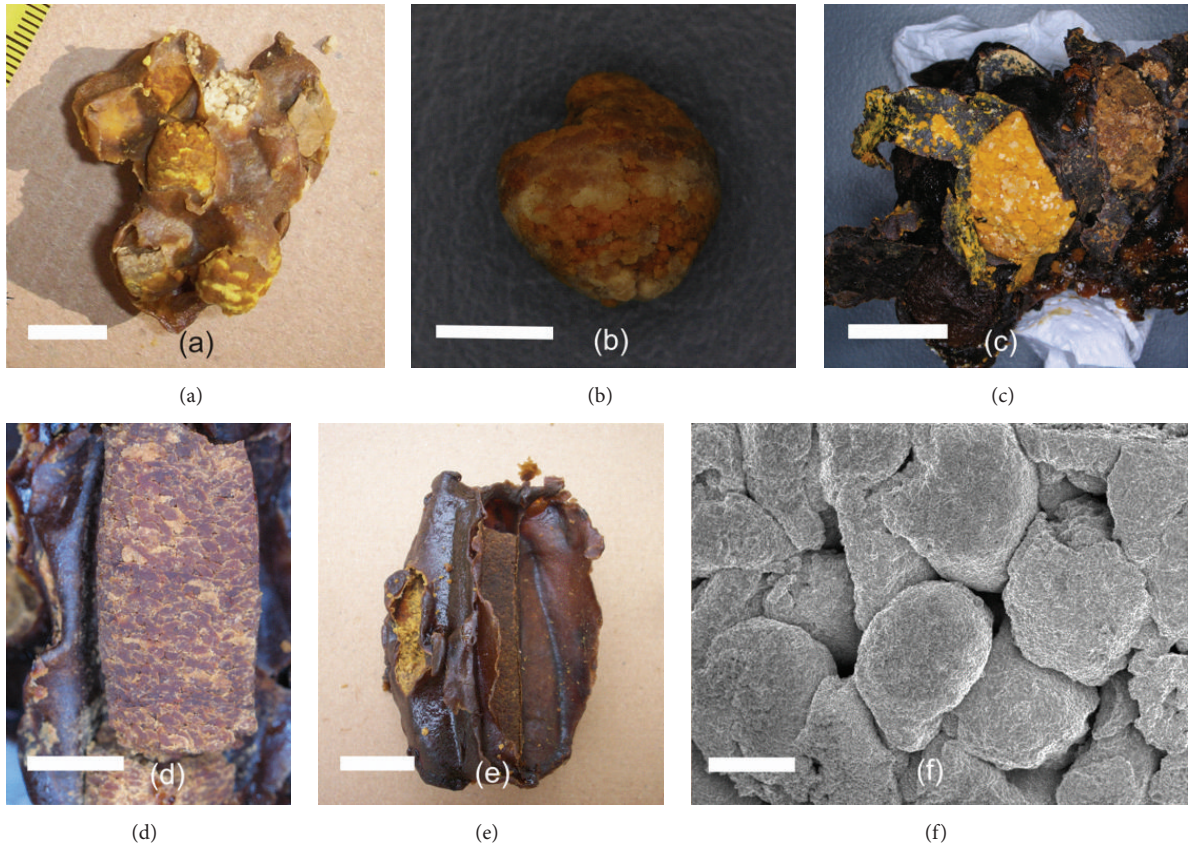

Figure 1: General aspect of pollen masses of *Tetragonisca fiebrigi* (a, b), *Melipona orbignyi* (c), and *Geotrigona argentina* (d, e). Individual loads from pollen masses can be seen in scanning microscope (f). Bars: (a) 10 mm; (b) 5 mm; (c) 20 mm; (d) 10 mm; (e) 20 mm; (f) 1 mm.

protein (Table 1). Higher protein values (>20%) were found in samples having the following composition: *Prosopis* (samples 10, 11, and 12 of *G. argentina*), type *Maytenus vitis-idaea* (samples 13 and 14 of *G. argentina*), type *Acacia praecox* + *Prosopis* (sample 7 of *M. orbignyi*), *Capparis speciosa* + *Mascagnia brevifolia* + Heliantheae (sample 5 of *T. fiebrigi*), *Ziziphus mistol* + *Prosopis* (sample 6 of *T. fiebrigi*), and *Castela coccinea* (sample 9 of *G. argentina*) (Table 1). The remaining samples showed less than 20% of crude protein; the lowest value (<10%) found was for type *Capparis tweediana-C. speciosa* (sample 8 of *G. argentina*).

Protein values found in samples composed mainly of herbs, climbers, or shrubs differed greatly among them (9.78–22.8%) and were slightly lower than those dominated by trees (17.84–30.41%). A broader range of protein values was found for *G. argentina* (9.78–30.41%) than for *T. fiebrigi* (17.84–22.80%).

4. Discussion

If protein content of pollen grains was constant for all individuals of each plant species, samples composed only or mostly of one pollen type (96–100%) (6 samples in the present study) would indicate their real protein value and it would be useful for comparing samples composed of many pollen types. Nutritive value of pollen grains is affected by air temperature, soil moisture, pH, and soil fertility, among other factors [30] but a range of values is expected for a particular species under the same conditions [16]. The two samples dominated by type *Maytenus vitis-idaea* had similarly high protein values (26.51 and 26.87%), followed by *Castela coccinea* that ranked near 20%, the minimum limit value considered as optimal to brood development in *Apis mellifera* L. [17], and the palm tree *Trithrinax schizophylla* with slightly less than 20%. The lowest protein value was from type *Capparis tweediana-C. speciosa* with less than half the optimal value. Inconsistency among pollen type composition and protein value was found for the three samples dominated by *Prosopis* as pure *Prosopis* pollen showed lower protein values than those composed of 91 and 89.5% *Prosopis* and approximately 10% of other types (9.2% *Ziziphus mistol* in sample 11 and 9.5% *Capparis salicifolia* + 1% *Ruprechtia triflora* in sample 12). It appears that these accompanying pollen types were providers of important amounts of proteins. Nevertheless, the differences of protein content detected in these three samples could be due to the fact that different species of *Prosopis* (similar at light microscope) were present in their composition, as many *Prosopis* species are highly abundant in arboreal and shrubby strata of the Chaco forest and meliponine bees forage on all of them. On the other hand, soil fertility can influence nitrogen

and consequent protein composition of plant individuals [30]. The dry Chaco is a xerophytic forest that alternates with water bodies such as ancient rivers and their related riparian vegetation [31]. Nests of meliponine species here studied were sampled from these environments, and bees could forage on flowers from both dry forest patches and water bodies composed of plants growing under different nitrogen level. Moreover, livestock grazing in the forest is a very common practice in the area studied and it is responsible for increasing soil nitrogen levels through excreta. Local differences of nitrogen levels in soil might be the cause for differences in protein amounts found in pollen masses dominated by a same pollen type (the case of *Prosopis*). Fertilizer incidence was discarded, as agricultural crops were absent in the sampled area.

Stored pollen in *Apis mellifera* colonies (pollen bread) is probably fermented by lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* from the honey stomach added to the pollen via regurgitated nectar [6, 7]. Over and above their significance in pollen bread production and storage, these lactic acid bacteria are important against pathogens and production and storage of honey [6, 9]. These microbial symbionts are also present in its ecologically similar and closely related group: the stingless bees (Meliponini) [9, 32]. Proteins, mainly enzymes, are secreted by this beneficial microbiota during stress [33], which could increase the protein content of stored pollen. Versatile digestive physiologies characterize broad polylectic bees [34], as pollen of diverse protein spectrum seems to be similarly foraged by these highly social species. Similar to other studies on bee foraged pollen [35, 36], protein content of pollen types was variable. It is likely that amino acids deficiencies of certain pollen are compensated by randomly foraging on a broad spectrum of pollen plants. This is in agreement with the argument stated by [37] that nutritive value of pollen for bees is not directly correlated with protein quantity since a qualitative factor is of greater importance. For instance, [38] detected low protein levels in hand-collected pollen from desert plants (from 7 to 15.6%), but they highlighted that their amino acid patterns were in agreement with the requirements for honey bees. An interesting topic to be tested by experimental studies is on the capability of meliponine brood to develop by eating pollen having the half of the optimal protein level for Apis mellifera. However, palynological surveys on meliponine bees from the Chaco region showed that scarce number of pollen masses are composed of pure or nearly pure pollen from *Capparis tweediana* or *C. speciosa,* having only 9.78% of protein (only six out of 75 masses analyzed of *G. argentina*, but none in 86 masses of *T. fiebrigi* or in ten of *M. orbignyi*) [39, FGV unpublished data]. Pollen with low protein levels would expose bees to more severe amino acids deficiencies. However, bees can be well developed when feeding on high amounts of these pollens, but a colony would be threatened when there is a low amount of pollen stores or shortage of flowerings [16]. Meliponine bees store great amounts of pollen (353 g in 20 pollen pots of diverse filling in one nest of *G. argentina*) [39] (Figure 1).

An attempt was made to determine the differences in protein content due to life-form of plants foraged and season when sampling was carried out and among bee species but further studies are necessary. However, most protein-rich pollen species were woody, in accordance with findings for other semiarid areas of Argentina [35] but findings by [36] did not show this pattern. It is widely known that *Solanum* species are protein-rich pollen resources, ranging from 34.1% for *S. tabanoense* Correll to 54.9% for *S. lycopersicum* L. [40]. *Solanum* was highly foraged by *Melipona orbignyi* [FGV unpublished data]. The only sample studied for *M. orbignyi* showed a protein value greater than the one required for *A. mellifera* and was dominated by types *Acacia praecox* and *Prosopis* followed by four types of less than 4% representation. As this species also prefers *Solanum* and other protein-rich pollen from poricidal anthers whose grains are easily gathered by vibratile buzzing, more samples would need to be analyzed to establish whether protein requirements are high for this *Melipona* species. The genus *Solanum* is well represented in the dry Chaco (more than 10 species), mainly in open areas of nitrogen-rich soils and water bodies.

It is surprising that pollen showing the highest protein content (>26%) belonged to highly nectariferous plants well represented in meliponine and *Apis* honey in this region such as *Prosopis*, *Maytenus*, and *Ziziphus* [39, 41–45]. Studies on protein content of pollen loads and pollen analysis of honey carried out in other semiarid areas also show this tendency, as seen in Table III of [35] and Table II of [46] for *Condalia*, Brassicaceae and *Prosopis*, in Table III of [36], and in Table II of [47] for *Adesmia*, Rosaceae, *Trifolium*, *Melilotus*, *Schinus*, and Brassicaceae. Furthermore, pollen from the nectariferous *Larrea* and *Prosopis* showed the richest protein value in a study carried out in a North American desert [38]. This pattern of high protein value in pollen of highly nectariferous plants foraged by honey-producing bees is here hypothesized and should be further studied.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The author thanks César Albornoz, Inocencio Medina, Isabel Brait, Rogelio Burgardt, Mercedes Koler, Ricardo "Nene" Vossler, and Juan Hiperdinger for their warm hospitality and help during the field studies in El Sauzalito, J. J. Castelli, El Espinillo, and Villa Río Bermejito and Nora Brea for providing suggestions and comments on the paper. The author is especially grateful to Arturo Roig-Alsina for identifying the bees. This study was supported by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).

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