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Sugar and protein content in different monofloral pollens - building a database

Ida CONTI[1], Piotr MEDRZYCKI[2], Chiara ARGENTI[2], Maria MELONI[2], Valentina VECCHIONE[2], Michela BOI[2], Mauro Giorgio MARIOTTI[1]
[1]*DiSTAV, University of Genova, Italy*
[2]*Council for Agricultural Research and Economics - Honey Bee and Silkworm Research Unit, Bologna, Italy*

Abstract

Pollen is the only protein source for the honey bee colony. Its nutritional quality varies according to the floral origin. The total protein content is a very important information in many research fields. Nevertheless its interpretation may be falsified by the sugars which are the main component of pollen pellets. In this paper we provide a database containing sugar and nitrogen content of 40 different pollen types.

Key words: pollen quality, protein content, sugar content, nutrition, corbicula, anther, honey bee.

Introduction

The scientific interest for the chemical composition of pollen has been increasing in the recent years, both for the growing application of pollen in human diet (Campos *et al*., 2010) and because nutritional stress is considered one of the most critical factors involved in the honey bee (*Apis mellifera* L.) health and colony losses (vanEngelsdorp *et al*., 2009; Naug, 2009; Brodschneider and Crailsheim, 2010). In fact, pollen nourishment can affect bee development (De Groot, 1953; Haydak, 1970) and their tolerance to pesticide intoxication (Renzi *et al*., 2016) and to some pathogens (Di Pasquale *et al*., 2013).

The chemical profile of pollen is widely variable according to the floral origin, thus it would be useful to know of the features of mono-typical pollens in order to: i) indicate to beekeepers the types of environment where to install apiaries, to obtain optimal pollen sources, both for the colonies and for commercial production; ii) build a pollen quality ranking, useful for the studies of its simple and synergistic effects on honey bees and man; iii) implement the guidelines for a sustainable management of seminatural- and agro-ecosystems in favour of pollinators.

The palynological analysis is based on morphological assessment of pollen grains and leads to the definition of "palynological types". In few cases they correspond to floral species, while usually only the genus or even family are determinable. Thus, "mono-typical pollen pellets" contain only one palynological type.

Protein content is often used as an indicator of the nutritional quality of pollen because: i) it influences several morphological, physiological and behavioural aspects in honey bees (Radev *et al*., 2014; Zheng *et al*., 2014; Frias *et al*., 2016); ii) proteins are the second most consistent compound in pollen pellets (Campos *et al*., 2008), making the latter a valid source of easily digestible peptides for humans (Campos *et al*., 1996).

In Roulston *et al*. (2000) the broadest collection of data about crude protein concentrations in anther pollen is available. These data are useful when studying the influence of this variable on honey bee foraging preferences, but they seem to be incomplete for nutritional studies. For honey bee and human nutrition, pollen pellets and beebread are used instead of anther pollen. Considering the objective difficulty to collect mono-typical samples of beebread, we focused on the pollen loads, whose carbohydrate and protein content are very similar to the former (Human and Nicolson, 2006).

Honey bees obtain corbiculae by collecting pollen grains from anthers, moistening them with glandular secretions and regurgitated nectar/honey, and packaging them on the hind legs (Casteel, 1912; Dadant, 1975). Thus corbiculae contain much higher quantities of sugar than pollen sampled directly from flowers. Moreover, Roulston *et al*. (2000) suggested that this quantity can vary between different pellet samples and thus we can't attribute a fixed value to the sugar added by foragers. Todd and Bretherick (1942) demonstrated also that reducing sugars may account for up to 41% of pellet's dry mass and that they are mostly attributable to the added nectar/honey. Notwithstanding, sugar components are often ignored in the research regarding pollen loads, or included in the total carbohydrates (containing also cellulose, starch and pectin not coming from the added nectar) often obtained in indirect way (e.g. Human and Nicolson, 2006).

This study was aimed to build a database of nitrogen/protein content and sugar profiles of pollen pellets belonging to botanical taxa commonly visited by honey bees principally in the area of Tigullio (Eastern Liguria, Italy). It was also our intention to estimate the nitrogen/protein content of the anther pollen, basing on the pellet analyses' results. The latter will be useful when it is impossible to analyse directly the anther pollen, due to insufficient quantity.

Materials and methods

Pollen pellets were collected using pollen traps in 7 sites and different periods (supplemental table S1). The large majority came from the Tigullio area in locations distributed in the basal and submontane belts, where the climate is humid-Mediterranean and the environmental systems surrounding the hives are characterised by several vegetational categories: urban areas with private gardens, olive groves, Mediterranean scrub, forests dominated by *Pinus pinaster*, *Quercus pubescens* or *Castanea sativa* and submontane grassland. The rest was collected in Emilia-Romagna region in order to include the taxa widely cultivated in this area.

Each sample was processed according to the following procedure: 1) the pollen pellets were separated in monochromatic groups; 2) to assure a 100% homogeneous floral origin of the monochromatic groups, each pellet was divided in two parts: one destined to the palynological assessment (Persano Oddo and Ricciardelli D'Albore, 1989) and one to build a cumulative mono-typical sample for the chemical analyses; 3) once obtained sufficiently big samples, each

was lyophilised for about 12 hours, powdered by a pestle and divided in two parts for the analyses of total nitrogen (N) and sugar content.

For the assessment of N, the Kjeldahl method (Bradstreet, 1954) was applied. A minimum quantity of 100 mg per sample was digested (400 °C - 4 hours) in strong sulphuric acid in presence of sodium sulphate and copper sulphate as catalysts. Then, after alkalinisation with 45% NaOH solution, the digest was steam-distilled and condensed in 4% boric acid solution. A direct titration with sulphuric acid N/20 was performed and the %N was calculated.

The analysis of sugars was carried out following the method of Szczesna (2007), adapted to our sample dimensions. At least 20 mg of each sample was dispersed in a small amount of deionised water and transferred to a 1 mL volumetric flask. 250 µL of methanol was added and brought up to notch with water. After 2 hours of rotary agitation, the suspension was passed through a 0.20 µm membrane filter and the filtrate was injected onto the column. The assessment of sugar content was carried out by HPLC, equipped with an isocratic pump, Refractive Index Detector (RID) and TEKNOKROMA Carbohydrate Column for polar phase (−NH2) (5 µm) 250 × 4.6 mm. The chromatographic separation was performed under the following conditions: flow rate: 1.0 mL/min, mobile phase: acetonitrile:water (75:25 v/v), column and detector temperature: 30 °C (± 1 °C), injection volume: 10 µL.

The estimation of %N in the anther pollen's dry mass (DM) was done by withdrawing, from the pellet sample's weight, the total sugars, whose great majority comes from the nectar/honey added by bees. To obtain accurate results, we should have considered the sugars naturally present in the anther pollen, but the relative information available in the scientific literature is limited. The only recent and most complete work (Tidke and Nagarkar, 2015) reported that, among the 15 studied species, the total carbohydrate content in anther pollen never exceeded 5.76% DM, with a mean of 3.47%. Considering that part of this mass is represented by several polysaccharides (cellulose, starch, pectin), the content of mono- and oligosaccharides should be nearly negligible or, in any case, much lower than 5%. Thus, deriving the %N in the anther pollen from the nitrogen and sugar content in the pellets is a good estimation with a low potential error.

Results and discussion

The contents of glucose, fructose and oligosaccharides, as well as N in mono-typical corbicular pollen, are given in supplemental table S2. The estimated %N in anther pollen is reported as well. For the discussion, in order to be consistent with the available literature, we converted all the mentioned data in protein content by applying the most commonly used factor of 6.25 (Roulston *et al*., 2000).

Total sugar content in our study ranged from 21.77 (*Zea mays*) to 58.95% DM (Apiaceae f. A < 25 µm). These data are consistent with what found by the other authors (Todd and Bretherick, 1942; Szczesna, 2007; Quian *et al*., 2008) except for 4 types which exceeded 50% value (Apiaceae f. A < 25 µm, *Linum*, *Ranunculus arvensis* and *Rubus* f.). In our research the mentioned minimum and maximum values of sugars corresponded to similar N contents in corbiculae, but the estimated N in anther pollen DM amounted respectively to 8.83 and 4.14% (> 2 fold). The protein content in the pollen pellet DM ranged between 13.8 and 30.4, which is close to the values obtained in most previous studies.

Analysing single genera, it is easy to observe that the data not always are coherent. For example the protein % in *Actinidia* pellets ranged from 15.4 (Liolios *et al*., 2015) to 18.1 (Tasei and Aupinel, 2008), which is definitely different from our result (27.1). The same authors found *Rubus* f. pollen containing 28.5 and 19.2% proteins respectively (24.2 in our study). Another example regards *Salix*, whose % protein content was 12.7 (Forcone *et al*., 2011), 14.8, 15.1 and 21.9 (Somerville, 2001), 15.4 (Todd and Bretherick, 1942) and 24.3 (our study). It might be thus hypothesised that the protein content in pollen pellets is not specific to the botanical genus but to the species.

Nevertheless, analysing the data regarding different pollen pellet samples of the same species we observed a similar situation. For example *Helianthus annuus* % protein content was found to account 12.9 and 13.8 (Somerville, 2001), 14.4 (Tasei and Aupinel, 2008), 16.4 (Taha, 2015) and 16.5 (our study). Similarly in *Z. mays*, the following protein % were found: 14.9 (Somerville, 2001), 16.6 (Liolios *et al*., 2015), 20.3 (this study). It seems thus that pollen pellets even of the same botanical species may vary in terms of protein content. In fact, Somerville (2001) analysed a consistent number of pollen pellet samples of 5 monospecific types (*Echium plantagineum*, *Eucalyptus bridgesiana*, *Hypochoeris radicata*, *Corymbia maculata* and *Rapistrum rugosum*) and found respectively the following % protein ranges: 28.1-37.4, 22.6-25.9, 9.2-18.2, 24.9-30.4 and 21.6-24.6. The differences within each type resulted statistically significant. This evidence suggests that it is not possible to attribute a fixed protein content value to the specific pollen pellets, contrarily to what happens for the anther pollen, as deduced from Roulston *et al*. (2000). The fact that the protein content in anther pollen is conservative within one botanical species (and even genus) and the same variable in pollen pellets is not, may be explained by the different mass contribution of the sugars added by bees during the process of pellet packaging. In fact, the bees use different nectar or honey sources, according to their availability (Hodges, 1952; Crane, 1990; Vaissiere and Vinson, 1994). Also Leonhardt and Blüthgen (2012) asserted that the sugar content in pollen pellets is not species-specific. This parameter depends both on factors related to the environment and season and on those linked to the pollen grain features. Among the latter ones we can evidence the pollen grain dimensions and weight but also the exine structure (Vaissiere and Vinson, 1994) and the pollenkit consistency (Klungness and Peng, 1983), which make agglutination and packaging more or less easy.

Conclusions

The protein content in pollen pellets can be characterised by a high variability even within one botanical origin, principally due to the unpredictable contribution of bee-added sugars. Thus, we propose a conversion method which allows to reduce this bias and to estimate, with an apparently good accuracy, the protein content in the anther pollen, which should be a conservative parameter.

We also suggest not to rely upon the nutritional facts of corbicular pollen available in the literature, generalising them to the botanical species, but to use the anther pollen values as the robust and stable ones. Their estimation, basing on the pellet composition, allows to overcome the technical prob-

lems related to the sampling and analysis of anther pollen.

The main objective of this study was to provide a database of protein content in the pollen of different botanical origins, but it would be interesting to continue and implement similar studies in order to add more species/pollen types to the database and to consider more nutritional facts (e.g. aminoacidic profile).

Thus, in answer to the problem of low reliability of pollen pellet composition, noticed by other authors (Roulston *et al*., 2000), the present study proposes a simple method of extrapolation of the anther pollen protein content, basing on the pellet protein and sugar composition. It should be also considered a beginning of a database of the so-obtained data, useful for the future studies.

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Authors' addresses: Piotr MEDRZYCKI (corresponding author, e-mail: piotr.medrzycki@crea.gov.it), Chiara ARGENTI, Maria MELONI, Valentina VECCHIONE, Michela BOI, Council for Agricultural Research and Economics - Honey Bee and Silkworm Research Unit, via di Saliceto 80, 40128 Bologna, Italy; Ida CONTI, Mauro Giorgio MARIOTTI, University of Genova - DiSTAV, corso Europa 26, 16132 Genoa, Italy.

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Authors: **Ida CONTI, Piotr MEDRZYCKI, Chiara ARGENTI, Maria MELONI, Valentina VECCHIONE, Michela BOI, Mauro Giorgio MARIOTTI**

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Table S1. GIS coordinates and altitude of the pollen sampling sites.

Site	Locality	Latitude (N)	Longitude (E)	Altitude (m above s.l.)
GE/1	Rapallo (GE)	44.3667	9.2166	196
GE/2	Chiavari (GE)	44.3267	9.3343	138
GE/3	Ne (GE)	44.3460	9.4405	420
GE/4	Vignale (GE)	44.3690	9.3416	300
GE/5	Bargagli (GE)	44.4347	9.0904	566
BO/1	San Giovanni in Persiceto (BO)	44.6320	11.2048	19
BO/2	Bologna (BO)	44.5239	11.3515	36

Table S2. Content (%) of mono- and oligosaccharides, as well as nitrogen (N) and proteins (derived by applying the 6.25 correction factor) in the mono-typical pollen pellets and anther pollen. (*) The N (and protein) content in the anther pollen is an estimation deriving from the %N in the pellet and corrected for its sugar content.
Note: samples 8 and 9 were collected in the same date and site but from different hives.

Sub-sample			Palynological type	Sugars (%)				Nitrogen (%)		Protein (%)	
ID	Date	Site		fructose	glucose	oligosaccharides	total	pellet	anther(*)	pellet	anther(*)
1	17 May 2015	GE/5	*Actinidia*	16.44	8.46	0.62	25.53	4.33	5.82	27.1	36.4
2	27 June 2016	BO/2	Apiaceae f. A < 25 µm	26.58	22.63	9.74	58.95	3.62	8.83	22.6	55.2
3	18 May 2014	GE/5	*Asphodelus albus*	15.32	10.27	1.95	27.54	3.79	5.24	23.7	32.7
4	11 July 2014	BO/1	*Brassica* f. > 25 µm	18.41	14.78	0.29	33.48	3.83	5.75	23.9	36.0
5	5 April 2015	GE/1	*Brassica* f. 20-25 µm	14.11	8.61	1.51	24.23	4.12	5.43	25.7	33.9
6	7 June 2015	GE/3	*Castanea sativa*	20.17	17.91	1.46	39.54	3.70	6.11	23.1	38.2
7	18 May 2014	GE/5	*Chamaerops* f.	20.06	13.01	0.42	33.49	3.57	5.37	22.3	33.6
8	19 July 2015	GE/2	*Cichorium* f.	23.75	16.76	8.82	49.34	2.67	5.27	16.7	32.9
9	19 July 2015	GE/2	*Cichorium* f.	21.28	15.69	7.09	44.06	2.52	4.50	15.7	28.1
10	18 May 2014	GE/5	*Cistus incanus* gr.	19.94	11.89	3.05	34.88	2.81	4.32	17.6	27.0
11	7 June 2015	GE/1	*Cistus monspeliensis* gr.	20.73	13.80	2.45	36.98	2.20	3.49	13.8	21.8
12	7 June 2015	GE/3	*Clematis*	19.08	16.67	0.87	36.63	3.33	5.26	20.8	32.8
13	19 July 2015	GE/2	*Convolvulus*	21.67	15.00	0.28	36.94	3.69	5.85	23.1	36.6
14	18 May 2014	GE/5	*Cornus sanguinea*	13.66	9.02	1.22	23.90	2.94	3.86	18.4	24.1
15	5 April 2015	GE/1	*Erica arborea* gr.	19.61	19.23	1.49	40.33	2.95	4.95	18.5	30.9
16	26 April 2015	GE/1	*Fraxinus ornus*	20.93	18.36	0.78	40.07	3.41	5.69	21.3	35.6
17	20 September 2015	GE/2	*Hedera*	14.83	16.68	3.75	35.26	4.04	6.25	25.3	39.0
18	27 June 2016	BO/2	*Helianthus annuus*	21.35	23.65	0.15	45.15	2.64	4.82	16.5	30.1
19	9 August 2015	GE/1	*Lagerstroemia*	14.28	9.26	4.55	28.09	3.70	5.14	23.1	32.1
20	19 July 2015	GE/2	Liliaceae 20-40 µm	19.35	16.45	0.18	35.99	3.26	5.09	20.4	31.8
21	7 June 2015	GE/3	Liliaceae 50-70 µm	16.92	12.68	1.09	30.69	4.22	6.09	26.4	38.1
22	19 July, 9 August 2015	GE/1-2	*Linum*	29.00	23.14	3.86	56.00	2.84	6.45	17.7	40.3
23	7 June 2015	GE/2	*Magnolia*	19.17	16.06	0.53	35.76	3.80	5.91	23.7	36.9
24	18 May 2014	GE/5	*Malus/Pyrus* f.	17.48	12.59	1.77	31.84	4.22	6.19	26.4	38.7
25	26 April 2015	GE/1	*Malus/Pyrus* f.	18.03	12.62	2.96	33.61	4.32	6.51	27.0	40.7
26	7 June 2015	GE/4	*Myrtus* f.	26.42	16.53	1.53	44.47	4.86	8.76	30.4	54.7
27	7 June, 19 July 2015	GE/2	*Oenothera* f.	17.33	15.12	1.05	33.49	2.71	4.07	16.9	25.5
28	7 June 2015	GE/3	*Olea*	20.33	19.40	0.58	40.31	2.68	4.48	16.7	28.0
29	7 June, 19 July 2015	GE/2	*Parthenocissus*	16.16	11.69	1.16	29.01	3.93	5.53	24.5	34.6
30	28 June, 19 July 2015	GE/2	*Phoenix*	22.03	17.76	0.16	39.95	3.30	5.49	20.6	34.3
31	27 June 2016	BO/2	*Plantago*	19.88	14.33	1.55	35.76	2.34	3.65	14.6	22.8
32	11 July 2014	BO/1	Poaceae < 37 µm	16.99	13.24	3.81	34.04	2.63	3.98	16.4	24.9
33	17 May 2015	GE/3	*Quercus ilex* gr.	26.93	14.52	0.34	41.80	3.36	5.78	21.0	36.1
34	26 April 2015	GE/1	*Quercus robur* gr.	20.33	15.80	1.04	37.16	3.45	5.49	21.6	34.3
35	18 May 2014	GE/5	*Ranunculus arvensis*	32.36	21.32	1.79	55.47	2.65	5.96	16.6	37.3
36	7 June 2015	GE/1	*Rosa* f.	21.20	19.16	2.60	42.97	4.14	7.25	25.8	45.3
37	7 June 2015	GE/3	*Rubus* f.	21.73	17.87	1.96	41.57	3.88	6.64	24.2	41.5
38	5 April 2015	GE/1	*Salix*	18.78	15.69	0.53	35.00	3.88	5.98	24.3	37.4
39	9 August 2015	GE/1	*Taraxacum* f.	15.49	8.77	6.98	31.23	2.94	4.27	18.4	26.7
40	27 June 2016	BO/2	*Zea mays*	11.13	10.65	0.00	21.77	3.24	4.14	20.3	25.9