DOI: 10.1515/JAS-2016-0028 J. APIC. SCI. VOL. 60 NO. 2 2016

DE GRUYTER OPEN

Original Article

FLORAL DIVERSITY OF POLLEN COLLECTED BY HONEY BEES (*APIS MELLIFERA* L.) – VALIDATION OF THE CHROMATIC ASSESSMENT METHOD

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Received: 12 September 2016; accepted: 6 November 2016

Abstract

Pollen collected from flowers by forager bees is the only natural protein source for the hive. This nutritional compound is fundamental for the nurse bee and brood development, and for the queen activity. Pollen has a strong influence on colony health. It is also known that the pollen quality, in terms of the amino acid profile and total protein content, varies significantly according to the floral origin. For this reason, the palynological diversity assessed in corbicular pollen is a good measure of the quality of the environment the bees live in, in terms of available food.

An international research initiative "C.S.I. Pollen" aims to elaborate a pollen diversity map for all of Europe, carried out by beekeepers. Chromatic assessment of pellet colours will be used as a method. In our study, we wanted to validate this idea, through the comparison between the two methods: chromatic assessment of the diversity of pollen pellet colours and palynological assessment of the real pollen type diversity. In other words, we wanted to verify whether the pellet-colour profile reflects the palynological one.

We found a significant correlation between results obtained from the two methods but some improvements are also proposed in order to increase the determination coefficient and to reduce the differences given by the two answers.

Keywords: chromatic assessment, corbicular pollen, floral diversity, honey bees, palynological analysis, pollen pellet colour

INTRODUCTION

Pollen is the main source of proteins, amino acids, minerals, fats, starch, sterols, and vitamins for a honey bee (*Apis mellifera* L.) colony (Stanley & Linskens, 1974; Herbert, 1992). The chemical composition of pollen varies considerably with the floral origin (Auclair & Jamieson, 1948; Roulston, Cane & Buchmann, 2000) and has important impacts on colony health. Pollen ingestion is necessary to develop different organs and tissues in adult bees (Haydak, 1970) such as fat bodies, ovaries, and hypopharyngeal glands. Moreover protein consumption rate is reported to be associated with adult bee longevity (Brodschneider & Crailsheim, 2010). Pollen is also important at the colony level because the quality of royal/worker jelly depends on the nurse bees' diet. Consequently, the latter may have a relevant impact on the morphology, behaviour, and physiology of all individuals (Brodschneider & Crailsheim, 2010), and accordingly on the quality of the pollination service (Scofield & Mattila, 2015). Pollen

nutrition also affects honey bee tolerance to some pathogens (Rinderer, Rothenbuhler & Gochnauer, 1974; Degrandi-Hoffman et al., 2010).

Survival and development of honey bee colonies, which are naturally associated with the availability of floral sources (notably pollen), depend in a relevant way, on the environmental management (Di Pasquale et al., 2013). Honey bees are commonly considered to represent the category of insect pollinators, because they occupy the same ecological niche. So, the former statement is also valid for the conservation of wild pollinators. The negative influence of low pollen quality in other bee species, like *Bombus terrestris* L., was also confirmed directly (Baloglu & Gurel, 2015).

The alteration of natural and semi-natural habitats, due to the development of intensive agriculture, produces two serious risks to bee nutrition: limited availability and low quality of pollen. The weed control and the loss of spontaneous plants lead to the reduction of pollen diversity and its availability periods. Moreover, humans obtained modern cultivars to intensify crop production, but the nutritional quality of pollen was not taken into account. Intensive agriculture is also an important source of toxic pollutants which the bees must deal with. The low nutritional quality of pollen, as well as its limited availability, may act as important factors negatively influencing bee resistance to pesticide intoxication (Schmehl et al., 2014).

The protein content in hand-collected pollen from flowers ranges from 2.5% to 61% (Roulston, Cane & Buchmann, 2000). The floral origin also influences the amino acid profile of pollen. In some cases, the latter may be inadequate compared to the nutritional requirements of honey bees. Among the ten essential amino acids, tryptophan (Weiner et al., 2010) and isoleucine (Somerville & Nicol, 2006), are often deficient in pollen, thus limiting colony development (de Groot, 1953). Furthermore, some pollen types collected by honey bees are known to be toxic to bees (Stanley & Linskens, 1974; Roulston & Cane, 2000). It is possible to hypothesise that when the pollen availability is limited to those species, it can have negative effects on bees.

A multifloral pollen diet is generally considered better than a monofloral one. In the former the risk of a lack of essential amino acids is lower and the effects of eventual toxic or suboptimal pollens are balanced by the presence of favourable ones (Eckhardt et al., 2014). Moreover, Alaux et al. (2010) found that a mixed pollen diet has a positive impact on the colony immunocompetence. Renzi et al. (2016) exposed bees to sub-lethal concentrations of thiamethoxam, and observed that those bees fed with a low pollen-diversity and protein-content diet developed smaller and irregular acini of the hypopharyngeal glands than the well-fed group. A polyfloral pollen diet may increase the lifespan of nurse bees infected with *Nosema ceranae* (Di Pasquale et al., 2013).

The importance of pollen diversity for solitary bees was also demonstrated. Haydak and Levin (1957) studied the larval diet of *Osmia lignaria* and found that in some cases pollen from only one plant species was not sufficient for complete development. Tasei and Masure (1978) investigated the factors affecting the larval development of *Megachile pacifica* and found that when the larvae were fed with a monofloral pollen diet, the development was slower than when they were fed with a bifloral one. Summarising, the diversity of available pollen can be considered a good estimator of the quality of the environment; an environment intended as trophic source for bees.

Considering the impact of the pollen diet on bee health, nutritional stress is one of the most relevant cofactors of CCD - Colony Collapse Disorder (Naug, 2009). Starvation and poor nutrition were indicated by US beekeepers, as two of the main causes of bee death (Van Engelsdorp et al., 2008). It is important to activate a large-scale monitoring survey of pollen availability and quality. To do it with the pure scientific criteria, an important financial investment would be necessary to support the apiary setting, the sample collection, and the chemical and palynological analyses. The international research initiative C.S.I. Pollen project,

in the framework of the COLOSS group (www.coloss.org), proposed an inexpensive way to investigate, throughout Europe, the diversity of pollen sources available to honey bees. It engages beekeepers as citizen scientists for the assessment of the chromatic diversity of collected corbicular pollen samples (Coloss, 2015). In addition to the scientific value of the project, the social aspect must also be mentioned. This aspect consists in intensifying the contact between scientists and beekeepers (i.e. local stakeholders). In 2014 and 2015, there were 465 and 585 participants, from 24 and 27 countries of the northern hemisphere, respectively (Brodschneider, Kalcher-Sommersguter & van der Steen, 2016).

The project assumes that the pollen chromatic diversity reflects the real floral diversity resulting from palynological analysis. In addition, the project assumes that the chromatic assessment carried out by beekeepers is comparable to the one observed by an expert in palynology. The first assumption may be in conflict with the not univocal association between colour and floral origin of pollen. The second assumption could prove problematic since there is a natural different perception of colours within a colour-normal population (Asano et al., 2016).

The objective of the present study was to validate the first of the main assumptions of the C.S.I. Pollen method, i.e. that the pellet colour diversity is a good estimator of the real diversity of bee-collected pollen.

To achieve this aim we proceeded by steps. First we compared the diversity of the pollen pellet colours with the diversity of the palynological profiles of the same samples to verify whether the two methods gave the same answer. Then we checked whether the results given by the two methods were at least correlated. To reduce the data variability and exclude error due to the subjective colour perception in different individuals, a laboratory chromatic assessment of all the samples was carried out by the same palynological specialist who was highly capable at distinguishing different pollen pellet colours.

ID	Colour	R	G	B	ID	Colour	R	G	B
Col. 1		0	0	0	Col. 16		255	215	0
Col. 2		38	51	74	Col. 17		255	165	0
Col. 3		74	25	44	Col. 18		237	118	14
Col. 4		94	33	41	Col. 19		252	62	2
Col. 5		117	21	30	Col. 20		220	156	0
Col. 6		142	64	42	Col. 21		175	117	5
Col. 7		204	6	5	Col. 22		159	80	9
Col. 8		211	110	112	Col. 23		117	75	25
Col. 9		244	219	170	Col. 24		146	50	0
Col. 10		250	250	210	Col. 25		157	145	1
Col. 11		255	255	209	Col. 26		198	196	100
Col. 12		255	216	127	Col. 27		202	196	176
Col. 13		255	255	151	Col. 28		126	123	82
Col. 14		240	240	140	Col. 29		53	124	45
Col. 15		248	243	53	Col. 30		80	78	0

Fig. 1. Colour palette used for the chromatic assessment of pollen pellets. The colour determination in RGB system is reported.

MATERIAL AND METHODS

An experimental apiary located in the Italian region of Liguria, Genoa Province, was identified. The site (Rapallo; lat: 44.3667N; long: 9.2166E) was chosen based on its high environmental diversity and richness, and the availability of pollen samples during the whole season. These factors allowed us to obtain both samples characterised by high and low palynological diversity. This suburban area was composed of mixed (deciduous and coniferous) forests, olive groves, home gardens, parks, and "green spaces" with ornamental plants.

Three healthy and queen-right hives (signed "a", "b", and "c") were chosen and equipped with pollen collection traps installed on the flight board. Corbicular pollen was collected from the traps, of each hive separately, between April and September 2015, following the C.S.I. Pollen protocol (C.S.I. Pollen Manual, 2015). The sampling dates are reported in Table 1.

From each pollen pellet sample, a 10g aliquot was randomly withdrawn and delivered to the CREA-API (Bologna, Italy) laboratory. It was divided into single-colour groups and the number of pollen pellets belonging to each group was counted by the specialist in the lab.

A standardised colour palette (Fig.1) was elaborated based on the palynologist's experience and previous publications (Hodges, 1952; Kirk, 2006). The colours were chosen so that each pollen pellet could be linked to a corresponding colour in the palette, even if this correspondence was not always exact. The palette was then printed in high quality to reduce the potential error due to different visualisation of colours by PC screens.

Subsequently, per each sample, the colour-groups were merged again, and powdered. Then, 2g of pollen dust were dispersed in 50mL of distilled water. The palynological analysis was carried out on aliquots of 0.01mL of this suspension. At least 1,000 grains for each slide were counted according to von der Ohe et al. (2004), properly adapted for pollen analysis. The numerical palynological profiles, defined according to Persano Oddo & Ricciardelli d'Albore (1989), were then converted to the volumetric ones to reflect real pollen mass instead of grain numbers (da Silveira, 1991). For this purpose, a database of average pollen grain volumes was produced based on the grain dimensions reported by the Ponet database (Ages, 2016).

Per each corbicular pollen sample, we obtained: 1) the pellet colour spectrum, and 2) the volumetric palynological profile.

Statistical analysis

The Shannon-Wiener diversity index (H') was calculated separately for both datasets (i.e. colour and palynological spectra). The two groups of the H' index values (chromatic vs palynological) were compared using the Wilcoxon matched pairs test.

Moreover, separately for each sample, the statistical comparison between the two index values was carried out applying the t-test. For this purpose, the method described in Magurran (1988), based on the procedure introduced by Hutcheson (1970), was followed.

In each sample, the total number (S) of pollen pellet colours, as well as the number of observed pollen types, were noted. The statistical difference between the two variables were assessed with the Wilcoxon matched pairs test.

Finally, for the diversity indexes and total colour numbers, the Pearson correlation between the chromatic and palynological data was investigated as well.

The Wilcoxon test and the Pearson correlation analysis were carried out using Statistica© (StatSoft Italia srl., 2005).

RESULTS

In the analysed samples, a total number of 90 palynological types and 28 pellet colours (out of 30 colours considered in the palette) were found overall.

The number of colour/palynological types (S) and the values of Shannon-Wiener index (H'), as well as the pairwise comparison results, are reported in Table 1. It is easy to notice that the difference in the diversity index values between chromatic and palynological assessment results

Table 1
Number of different pellet colours/palynological types (S) and values of Shannon-Wiener diversity index (H'), assessed by two different methods: chromatic assessment of pellet colours and palynological analysis

sample ID	date	hive ID	S		H'				
			chromatic	palyno-logical	chromatic (variance[1])	palynological (variance[1])	t[1]	df[1]	p
S1	2-5 April	a	11	22	1.68 (0.001)	2.12 (0.001)	9.5	1794	<0.001
S2	2-5 April	b	12	26	1.77 (0.001)	2.37 (0.001)	14.3	1849	<0.001
S3	23-26 April	b	12	17	0.83 (0.001)	1.21 (0.001)	8.1	2146	<0.001
S4	14-17 May	a	13	19	1.17 (0.001)	2.06 (0.001)	19.3	2242	<0.001
S5	14-17 May	b	2	15	0.67 (0.000)	1.21 (0.002)	11.8	1048	<0.001
S6	4-7 June	a	10	23	1.61 (0.001)	2.10 (0.001)	12.3	1932	<0.001
S7	4-7 June	b	8	20	1.32 (0.001)	1.81 (0.001)	10.8	1990	<0.001
S8	4-7 June	c	8	27	1.27 (0.000)	1.59 (0.002)	6.3	1345	<0.001
S9	25-28 June	a	4	4	0.76 (0.000)	0.88 (0.000)	5.5	1980	<0.001
S10	25-28 June	b	4	2	0.68 (0.000)	0.51 (0.000)	7.6	1977	<0.001
S11	25-28 June	c	6	5	0.82 (0.001)	0.65 (0.001)	5.3	2176	<0.001
S12	16-19 July	a	10	20	0.80 (0.001)	0.81 (0.002)	0.0	1958	0.995
S13	16-19 July	b	12	10	0.65 (0.001)	0.74 (0.002)	1.6	2239	0.102
S14	16-19 July	c	6	19	0.96 (0.001)	1.12 (0.002)	2.8	1773	0.005
S15	6-9 August	a	11	18	1.36 (0.001)	1.93 (0.001)	12.7	2220	<0.001
S16	6-9 August	b	7	18	1.02 (0.001)	0.69 (0.002)	7.0	1601	<0.001
S17	6-9 August	c	10	25	1.61 (0.001)	1.44 (0.002)	3.4	1541	<0.001
S18	17-20 September	a	2	2	0.65 (0.000)	0.01 (0.000)	56.7	2025	<0.001
S19	17-20 September	b	3	4	0.97 (0.000)	0.06 (0.000)	45.7	1845	<0.001

[1] t statistic, df (degree of freedom) and variance were calculated according to Hutcheson (1970)

was almost always significant.

By processing the entire data set, the results clearly show that there was no statistical difference in the H' values between the two ways of diversity assessment, even if the mean number of types (S) was significantly different (Table 2).

When analysing the Pearson correlation between the data obtained through the pellet colour assessment and the palynological analysis, in terms of the number of types (S) and the Shannon-Wiener diversity index (H'), we can observe in both cases, a significant correlation ($p<0.05$) and a coefficient of determination (R^2) around 0.5 (Figs 2 and 3). The index conversion formulas are also proposed (regression formula). These should be useful when we have the results of the chromatic assessment of pollen pellet colour-diversity, and when we need to foresee the real diversity of pollen types without carrying out a palynological analysis. Nevertheless, these formulas were elaborated based on 19 analysed samples only. To give the formulas more reliability and statistical strength, this study should be followed by other studies with similar objectives. More such studies would significantly increase the dataset and strengthen the conclusions.

Table 2

Overall mean of the number of pellet colours/palynological types (S) and values of Shannon-Wiener diversity index (H'), assessed by two different methods: chromatic assessment of pellet colours and palynological analysis

variable	mean		results of Wilcoxon matched pairs test		
	chromatic	palynological	N	Z	p
H'	1.08	1.23	19	1.288	0.198
S	7.95	15.58	19	3.219	0.001

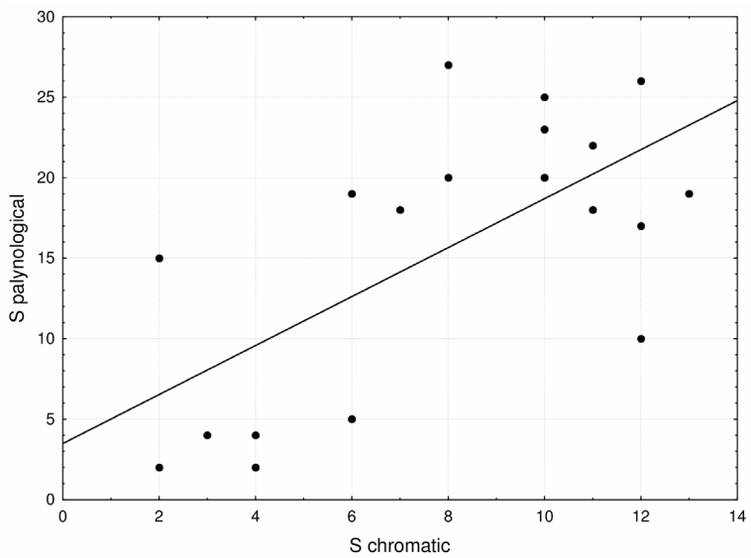

Fig. 2. Relationship between chromatic and palynological assessment in terms of the total number of observed types (S).
The results of the correlation: R^2= 0.44; p = 0.002; S palynological = 3.49 + 1.52 * S chromatic

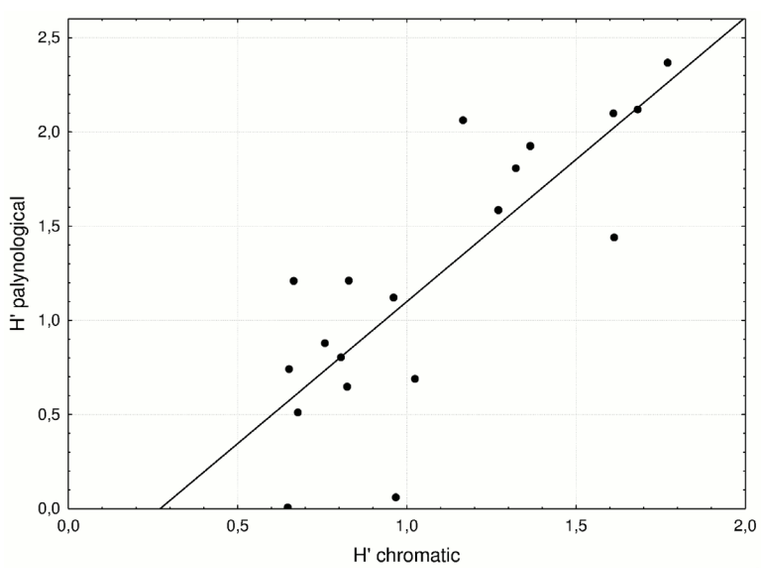

Fig. 3. Relationship between chromatic and palynological assessment in terms of the value of Shannon-Wiener index (H').
The results of the correlation: R^2= 0.66; p < 0.001; H' palynological = -0.41 + 1.51 * H' chromatic

DISCUSSION

While observing the results of all the samples pooled in one statistical analysis (Table 2), it might be noticed that the values of the Shannon-Wiener index (H') calculated for the palynological analyses didn't differ significantly from the same index calculated for the chromatic profiles of pollen pellets. This might lead to a false conclusion that the chromatic assessment could serve as a good estimator of the real pollen diversity in the analysed samples. It is important to note, that applied statistics (in this case Wilcoxon matched pairs test) cannot "recognise" the nature and structure of the compared variables. In fact, when the same comparison was carried out separately for each pair of data using the specific t-test, designed appropriately for the comparison of two H' indexes (chromatic *vs* palynological diversity), in 17 out of 19 analysed samples (i.e. 89%), it turned out that the difference between the two assessment methods were statistically significant (Table 1). Also, the total number of observed pellet colours in the overall analysis was significantly different from the total number of palynological types.

The correlation between the results obtained by the two methods was statistically significant. But the level of the coefficient of determination (R^2) does not allow us to make statements about a very strong relationship between the two variables.

We can assert, that the chromatic assessment of pollen pellets' diversity, when carried out by an expert with a "sensitive eye", can be a good tool, useful for the estimation of the real diversity of palynological types that bees have access to. This means that the basic idea of the C.S.I. Pollen initiative seems to be built on a solid basis. Nevertheless, important improvement would be desirable to enhance the reliability of this method. We identified two principal aspects to be discussed and studied in future research work.

1. Coefficients of correction for each pellet colour

It is evident that the number of different pellet colours does not correspond to the number of palynological types. In fact, it is known that flowers from different plant species may give pollen pellets of the same colour, and also that flowers of one plant species may give pollen pellets of different colours (Simonetti et al., 1989). It is also clear that the number of palynological types corresponding to one pellet colour is not fixed but changes according to the colour. For this reason, a hypothetical situation where we have observed yellow, orange, green, and ochre pellets (which normally correspond to a high number of different flower species) will have a completely different meaning than another one with violet, black, light-pink, and blue pellets (each of these colours corresponding to a few species), even if in both cases the total number of colours was 4. It is clear that the former sample may be characterised by a definitely higher palynological diversity than the latter. A database of colour correction ratios would complete the C.S.I. Pollen method and make it more reliable.

To discuss some real examples, in our study the sample S5 presented only 2 pellet colours (gold - Col.16 and orange - Col.17), but the palynological analysis discovered 8 pollen types exceeding 1% of a total pollen mass. Seven of those 8 correspond to the two mentioned colours. This different response given by the two assessment methods was also seen in the H' values (see Table 1).

On the contrary, the sample S18 was composed completely of *Hedera* sp. (>99%) but two pellet colours were identified in an almost equal quantity: salomie - Col.12 and orange - Col.17. This means that both colours are characteristic of this botanical species.

The sample S9 is a rare example of direct correspondence between pellet colour and pollen botanical origin. No yellow nor orange pellets were identified and each of all the 4 colours could be directly related to one of the palynological types.

Therefore, we propose a specific study aimed to elaborate a list of correction ratios per different pellet colours to reduce the error given by the unequal number of floral species corresponding to different colours. We think that by applying these ratios, the correlation between the

chromatic and palynological data will reach a high level and the pairwise difference between diversity indexes calculated with the two methods will be reduced.

2. Individual differences in colour perception and improvement of thresholds for the definition of abundance categories

The C.S.I. Pollen project is not based on chromatic assessment carried out by a specialist, but on a simplified method applied by the beekeepers, who take care of the apiaries. It would be wise, though, to validate the other phases of the method (the beekeepers' visual assessments, the thresholds for the attribution of colour abundance categories).

Summarising, we consider the C.S.I. Pollen programme to be an interesting tool, based on a reliable method. The programme has the potential of providing huge amounts of data with a minimum of expense. Also the social aspect of the entire initiative cannot be ignored. Nevertheless, to improve the quality of the results obtained through this project, a number of improvements would be necessary.

ACKNOWLEDGEMENTS

The authors would like to thank the beekeeper association Nuova Assoapi Ligure, and Arturo Assereto - the beekeeper who volunteered to participate in this project by managing the bee hives and collecting pollen pellets at regular intervals.

REFERENCES

Ages. (2016). Ponet database, from http://ponetweb.ages.at/pls/pollen/pollen_suche

Alaux, C., Ducloz, F., Crauser, D., Le Conte, Y. (2010). Diet effects on honey bee immunocompetence. *Biology Letters, 6*, 562-565. DOI:10.1098/rsbl.2009.0986

Asano, Y., Fairchild, M. D., Blondé, L., Morvan, P. (2016). Color matching experiment for highlighting interobserver variability. *Color Research & Application, 41,* 530–539. DOI:10.1002/col.21975

Auclair, J. L. & Jamieson, C. A. (1948). A qualitative analysis of amino acids in pollen collected by bees. *Science, 108*, 357–358.

Baloglu, G. H. & Gurel, F. (2015). The effects of pollen protein content on colony development of the bumblebee, Bombus terrestris L. *Journal of Apicultural Science, 59(1), 83-88.* DOI: 10.1515/JAS-2015-0009

Brodschneider, R. & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie, 41*, 278-294. DOI:10.1051/apido/2010012

Brodschneider, R., Kalcher-Sommersguter, E. & van der Steen, J. (2016). Overview of two years C.S.I. Pollen. In *Proceedings of C.S.I. Pollen project workshop* (p. 7). Glasgow. Scotland.

Coloss. (2015). C.S.I. Pollen, from http://www.coloss.org/taskforces/csi

C.S.I. Pollen Manual (2015). C.S.I. Pollen: How to investigate pollen diversity available for honey bees, from http://www.irishbeekeeping.ie/index.php/csi-pollen-project

Da Silveira, F. (1991). Influence of pollen grain volume on the estimation of the relative importance of its source to bees. *Apidologie, Springer Verlag, 22*(5), 495-502.

DeGrandi-Hoffman, G., Chen, Y., Huang, E., Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). *Journal of Insect Physiology, 56*(9), 1184-91. DOI:10.1016/j.jinsphys.2010.03.017.

De Groot, A.P. (1953). Protein and amino acid requirements of the honey bee (Apis mellifica L.). *Physiol. Comp. Oecol., 3,* 197–285.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J. L., Alaux, C. (2013). Influence of Pollen Nutrition on

Honey Bee Health: Do Pollen Quality and Diversity Matter?. *PLoS ONE, 8*(8), e72016. DOI:10.1371/journal.pone.0072016

Eckhardt, M., Haider, M., Dorn, S., Müller, A. (2014). Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? *Journal of Animal Ecology, 83*, 588–597. DOI:10.1111/1365-2656.12168

Haydak, M.H. (1970). Honey bee nutrition. *Annual Review of Entomology, 15*, 143-156.

Herbert, E. W. J. (1992). Honey bee nutrition. In Graham, J. E. *The hive and the honey bee*. (pp. 197–233). Hamilton: Dadant & Sons Inc.

Hodges, D. (1952). The pollen loads of the Honeybee. London: Bee Research Association Limited

Hutcheson, K. (1970). A test for comparing diversities based on the Shannon formula. *Journal of Theoretical Biology, 29*(1), 151-4.

Kirk, W. D. J. (2006). A colour guide to pollen loads of the honey bee. Cardiff: IBRA

Levin, M. D. & Haydak, M. H. (1957). Comparative value of different pollens in the nutrition of Osmia lignaria. *Bee World, 38*(9), 221-226

Magurran, A. E. (1988). *Ecological diversity and its measurement.* (pp. 179). London: Chapman and Hall

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honey bee colony collapses. *Biological Conservation, 142*, 2369–2372. DOI : 10.1016/j.biocon.2009.04.007

Persano Oddo, L. & Ricciardelli d'Albore, G. (1989). Nomenclatura melissopalinologica. *Apicoltura, 5*, 63-72.

Renzi, M.T., Rodríguez-Gasol, N., Medrzycki, P., Porrini, C., Martini, A., Burgio, G., Maini, S., Sgolastra, F. (2016). Combined effect of pollen quality and thiamethoxam on hypopharyngeal gland development and protein content in *Apis mellifera. Apidologie* DOI:10.1007/s13592-016-0435-9

Rinderer, T. E., Rothenbuhler, W. C. & Gochnauer, T. A. (1974). The influence of pollen on the susceptibility of honey-bee larvae to Bacillus larvae. *Journal of Invertebrate Pathology, 23*, 347-350. DOI:10.1016/0022-2011(74)90100-1

Roulston, T., Cane, J. H. & Buchmann, S. L. (2000). What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny?. *Ecological Monographs, 70,* 617–643.

Schmehl, D. R., Teal, P. E. A, Frazier, J. L., Grozinger, C. M. (2014). Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (Apis mellifera). *Journal of Insect Physiology, 71*(0), 177-190. DOI: 10.1016/j.jinsphys.2014.10.002

Scofield, H. N. & Mattila, H. R. (2015). Honey Bee Workers That Are Pollen Stressed as Larvae Become Poor Foragers and Waggle Dancers as Adults. *PLoS ONE, 10*(4), e0121731. DOI:10.1371/journal.pone.0121731

Simonetti, G., Frilli, F., Barbattini, R., Iob, M. (1989). Flora di interesse apistico. Uno studio di botanica applicata in Friuli-Venezia Giulia. *Apicoltura, app., 5*, pp. 377

Somerville, D. C. & Nicol, H. I. (2006). Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity. *Australian Journal of Experimental Agriculture, 46*, 141 –149

Stanley, R. G. & Linskens, H. F. (1974). *Pollen: Biology, biochemistry, management.* Heidelberg: Springer. DOI: 10.1007/978-3-642-65905-8

StatSoft Italia srl. (2005). STATISTICA (software for the data analysis), version 7.1., www.statsoft.it

Tasei, J. N. & Masure, M. M. (1978). Sur quelques facteurs influençant le développement de Megachile pacifica Panz. (Hymenoptera, Megachilidae). *Apidologie, 9*(4), 273-290

Van Engelsdorp, D., Hayes, J. Jr., Underwood, R. M., Pettis, J. (2008). A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLOS ONE, 3,* e4071. DOI:10.1371/journal.pone.0004071.

von der Ohe, W., Oddo, L., Piana, M., Morlot, M., Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie, Springer Verlag, 35* (Suppl. 1), S18-S25. DOI:10.1051/apido:2004050.

Weiner, C.N., Hilpert, A., Werner, M., Linsenmair, K. E., Blüthgen, N. (2010). Pollen amino acids and flower specialisation in solitary bees. *Apidologie, 41,* 476 –487

Annex 1 - Results of the palynological analyses, expressed in volumetric percentage

Palynological type	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
Acer		1.00																	
Actinidia				0.88	0.12														
Aesculus			0.10		0.11														
Agave												0.54							
Ailanthus						1.12	0.71	0.94											
Allium f.	1.80	4.55	1.72	2.95															
Anemone	3.93	0.41	0.47																
Apiaceae f. A <25µm				0.02															
Apiaceae f. A >25<35 µm	0.11	0.77						0.03											
Arctium												0.37		0.65					
Asparagus acutifolius																0.03			
Asteraceae f. A							0.13												
Asteraceae f. H Aster t.	1.42	0.23	0.16			0.06						0.24		0.31	0.29	0.02	0.29		
Asteraceae f. H Helianthus t.																0.19	0.28	0.10	
Asteraceae f. T	14.40	4.29		0.20	0.31							2.11	1.49	3.29	0.50	84.03	19.07		
Borago		0.19	0.52					0.26				0.78							
Brassicaceae	2.03	5.72	0.20																
Camellia		0.90																	
Carex	0.46	0.95																	
Caryophyllaceae																	0.57		
Castanea						0.37		1.56	52.48	79.23	74.41	0.04	0.30	0.01	0.99		0.01		
Centaurea jacea															7.14	0.33	0.86		0.12
Centranthus ruber																	0.71		
Cephalaria												3.07			19.54	9.73	1.05		
Chamaerops			0.46	2.87	1.13														
Chenopodiaceae								0.83						0.07			0.38		
Cistus incanus gr.								0.58											
Cistus monspeliensis gr.						1.92		0.46											
Citrus sinensis												2.23	1.85	1.25	0.21				
Citrus limon			0.30	0.56		1.26	0.82												
Clematis												0.07	0.44	0.06	2.82		0.30		
Commelina						0.19		0.10				0.20	0.20	0.04					
Cornus sanguinea				15.29															
Diospyros								0.43											
Echinops												1.16							
Erica arborea	36.50	13.46	1.07																
Eucalyptus				1.28	3.45	1.06	2.28	0.81	5.59		0.38								
Fabaceae others						1.06	0.66	0.19											
Fragaria/Potentilla							0.46												
Fraxinus			17.22	30.96	71.27														
Genista f.												0.60			0.24	0.36	1.03		
Hedera																		99.90	99.06
Ilex			0.10																
Juglans	0.23							1.12											
Lagerstroemia													0.52	0.89	26.93	1.22	59.24		
Laurus	1.30	8.58																	
Ligustrum f.						0.22		0.31				0.23	1.18	1.01					
Linum																1.32	1.91		

Liriodendron				1.62													
Lonicera						4.32		3.08									
Lotus			0.02														
Magnolia												6.93	16.36	1.37		1.77	
Malus/Pyrus f.	8.79	25.68	19.21														
Melilotus						0.15							0.07			0.03	
Mentha f.															0.03		
Mercurialis	0.32	0.17	0.04								0.06		0.55	0.05	0.58		
Ocimum																0.46	
Odontites																	0.69
Olea				6.00	4.10	23.85	0.78	1.10							0.08	0.07	
Ornithogalum t.	1.11	0.97					2.35	0.33									
Ostrya	11.45	2.59															
Oxalis		0.30															
Palmae														13.66		0.10	
Parthenocissus							0.41	0.17			0.78		0.45	3.52	0.62	0.54	
Pinus		0.55		1.06	1.67												
Plantago							0.67	0.19			2.20	4.15	1.91	0.69		0.43	
Poaceae						0.39	1.48	0.14							0.17		
Polygonum bistorta																0.98	
Prunus f.	8.94	18.53			0.50												
Psoralea														0.27			
Puccinia								0.06			0.09		0.07			0.06	
Quercus ilex	0.05			10.18	7.97	20.21	29.32	9.73							0.04		
Quercus others	1.69	0.44	58.06	4.21	4.79	7.42	10.73	1.80									
Ranunculus	0.82	4.31	0.25														
Rhamnus	1.19	0.67															
Robinia				0.67	0.53												
Rosa f.					0.98	16.75	33.75	56.02									
Rosmarinus		0.84															
Rubus f.				0.32		16.53	13.37	18.45	41.67	20.77	23.84	84.52	82.94	70.22	21.59	0.61	9.30
Rumex						0.28											
Salix	3.41	3.53															
Sambucus nigra	0.05	0.32	0.10	0.27		0.13	0.07	0.16									
Schinus							0.06		0.26		0.36						
Scilla t.						0.14	0.24					0.15		1.07	0.12		
Solanum dulcamara	0.01	0.02															
Thymus f.				20.14	2.42										0.33		0.12
Trifolium repens gr.				0.51	0.65	1.13	1.37	0.88			1.02	0.55		1.71	0.09	0.34	
Unknown 1						0.09											
Verbascum f.															0.32	0.23	
Vitis						1.35	0.34	0.29									

Annex 2 - Results of the chromatic assessment of the samples, expressed as the percentage of pollen pellets belonging to each colour category

ID	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
Col. 1		0.08													0.65		0.24		
Col. 2																			
Col. 3				0.08			0.08												
Col. 4	0.25		0.08								0.40		0.29						
Col. 5																	0.81		
Col. 6								0.25					0.74	5.10			2.19		
Col. 7															0.08	0.23			
Col. 8	1.72		0.67	24.19								0.90	0.37						
Col. 9		2.15	0.17	2.13		0.08						0.45	1.99		1.47				
Col. 10				0.08															
Col. 11				0.95															
Col. 12	29.56					3.06			0.92				2.95			44.73		34.96	
Col. 13											0.57								
Col. 14	9.85	1.04	0.92									3.98		16.94		2.11			
Col. 15	8.25	0.48										0.98			2.21				
Col. 16		12.76		0.24	38.24								1.40						57.59
Col. 17		19.14	0.58	5.28	61.76	22.38		31.24			63.72	0.98			1.55			65.04	16.95
Col. 18	13.18			0.24		0.53				0.18		0.45	0.66	6.75		46.84	23.80		25.45
Col. 19	32.88	4.23	0.25			4.05		30.15							0.25		1.46		
Col. 20			74.35	62.02		37.66	3.06	0.17				8.03		0.64	59.93		21.69		
Col. 21			0.58	2.13		0.69	56.08		53.93	64.41		2.03	1.99	0.89		4.68	2.19		
Col. 22				1.02				2.26	0.38				0.88				35.74		
Col. 23	3.33	28.71	0.08	1.34		2.06	16.30	0.84		0.26	2.19				12.02				
Col. 24	0.25	3.19				7.10	3.06									0.62			
Col. 25	0.37		3.33	0.32									0.88			0.78			
Col. 26	0.37	26.56	18.90					0.50			32.39								
Col. 27							2.07								8.75				
Col. 28		1.44	0.08			22.38	16.30	34.59	44.77	35.15	0.73	0.98	87.41	69.68	10.87		10.80		
Col. 29																			
Col. 30		0.24					3.06					81.25	0.44		2.21		1.06		