

Original Article

POLLEN FORAGING BY HONEY BEES (*APIS MELLIFERA* L.) IN GREECE: BOTANICAL AND GEOGRAPHICAL ORIGIN

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Abstract

Pollen is very important for honey bee colony development and nutrition. It is also a valuable product for human consumption, considered to have high nutritional value. In this study, we performed melissopalynological analysis of 285 pollen load samples collected from 44 apiaries throughout Greece. The analysis revealed 229 plant taxa represented in total. The abundance of each pollen type varied among the geographical areas from which the samples were collected. We also observed variation among samples collected from the same geographical region. The most frequently found families were Fabaceae, Asteraceae and Rosaceae. The most frequently observed taxa were Brassicaceae, *Carduus* type, *Cistus* and *Papaver rhoeas*. Statistical analysis showed that the geographical classification of pollen samples among northern, central and southern Greece is possible.

Keywords: *Apis mellifera*, botanical origin, geographical origin, Greece, melissopalynology, pollen.

INTRODUCTION

Honey bees depend on nectar and pollen to sustain themselves. Pollen provides lipids, carbohydrates, vitamins, and minerals to the colonies; and it serves as the honey bees' only protein source (Roulston and Cane, 2000). Pollen is necessary for brood and young worker development, thus, colony survival and growth depend on the quantity and the nutritional quality of the pollen resources provided by the foragers. The shortage of pollen can have significant effects, both on the strength and resilience of the colony and on honey production (McLellan, 1974). Nutritional quality, as well as quantity, is important. Pollen containing less than 20% crude protein cannot satisfy the colony requirements for its development and optimum honey production (Herbert, 1992). Pollen collected from different plant species has different nutrient value and may differ significantly in amino acid composition and/or concentration (Day et al., 1990; Roulston et al., 2000). Thus,

knowledge of the composition and diversity of the pollen sources within an area is very important to the local beekeeping industry.

Pollen is also considered to be a product of high nutritional value for human consumption. During recent years, its demand has been continually increasing in the world market (Villanueva et al., 2002). Because the composition of commercial pollen varies greatly depending on the botanical and geographical origin (Linskens and Jorde, 1997), the proper labeling of the product for human use necessitates an understanding of these parameters.

Greece is located in the center of the eastern Mediterranean, at the junction of Africa, Asia, and Europe, creating a habitat with a rich and diversified flora (Phitos et al., 1995). The rich flora and territorial diversity, along with the abundant sunshine, create a unique environment for the development of apiculture. Beekeeping plays a very important economic role; about 20,000 families in Greece maintain over 1.5 million honey bee colonies (Papa-

panagiotou, 2010). Although several studies have addressed nectar sources and honey types produced in Greece (Thrasylvoulou and Manikis, 1995; Tsigouri et al., 2004; Dimou et al., 2006b; Karabournioti et al., 2006; Karabournioti et al., 2009), there are few studies about the pollen flora in Greece to date (Dimou and Thrasylvoulou, 2007a,b).

The aims of this study are: to identify the floral composition of bee pollen in main apicultural areas in Greece and to collect and provide botanical and geographical information relevant to the potential marketing of bee pollen as a commercial product.

MATERIAL AND METHODS

Pollen samples were collected from pollen traps fitted in the entrance of honey bee (*Apis mellifera* L.) hives during 2009 - 2012. The traps remained closed for at least three days per week and the pollen was collected weekly, as suggested by Dimou et al. (2006a). Pollen was obtained during spring and early summer (April to June), which is the main pollen season in Greece (Dimou et al., 2006a; Dimou and Thrasylvoulou, 2007a). The pollen load samples were collected from 44 apiaries corresponding to 16 prefectures of main apicultural interest in Greece. The sites varied in surrounding flora, elevation, temperature, and rainfall. The great

majority of the apiaries were located in areas near both natural habitat and agricultural landscape with crops such as wheat, maize, cotton, and olive trees. All of the beekeepers who participated in this work were members of the "Hellenic Association of Royal Jelly and Other Bee Products Producers." In total, 285 pollen samples were processed: 111 samples were collected from northern Greece (Thessaloniki, Pieria, Chalkidiki, and Kilikis), 96 from central Greece (Arta, Viotia, Larisa, Phthiotis, Magnesia, Attiki, Achaia, Kefallinia, and the Cyclades islands of Tinos and Paros), and 78 from southern Greece (Chania, Lasithi, and Heraklio) (Fig. 1).

At least 300 pollen loads from each sample were sorted by color, and at least two slides of each color class were prepared without acetolysis, using similar methodology as described by Louveaux et al. (1978). Each examined pollen load was placed on a slide with a drop of a glucose solution and stained with alcoholic fuchsine. The slide was then dried by slight warming (not above 40°C) and mounted with Entelan™ (Entelan Microscopy, Karlsruhe, Germany). At least 500 pollen grains on each slide were counted under the microscope. If at least 95% of the counted pollen grains on a slide were identified to be the same type, it was considered that this pollen load was from a single floral source.



Fig. 1. Prefectures of Greece from which the pollen samples were collected.

To determine the botanical classification of the taxa from which the pollen was derived, both color and grain morphology of the pollen load were considered (Hodges, 1974; Dimou and Thrasylvoulou, 2007b). Pollen loads with the same pollen type and color were segregated and weighed to quantitate the contribution of each individual pollen source. The following classes were defined to describe the frequency with which a botanical source is represented within a given pollen load: "class I", rare, found in <3%; "class II", very few, found in 3 - 15%; "class III", few, found in 16 - 45%; and "class IV", frequent, found in >45%. When a pollen load was derived from more than one botanical source, the grouping within the above classes was assigned to be proportional to the counted percentage of the pollen grains on the slides. However, such cases were very rare, and they concerned only taxa found in <3% in the sample. For the microscopic identification of the pollen types, the collection of reference slides from the Laboratory of Apiculture of the Aristotle University of Thessaloniki and pollen atlases were used (D'Albore, 1998; Moshidis et al., 2013).

The Diversity Index of Shannon and Weaver (1949) was used to indicate the pollen type diversity and species richness:

$$H' = -\sum_{i=1}^n p_i \cdot \ln p_i,$$

where H' is the Shannon-Weaver Diversity Index, and p_i is the proportion of each pollen type i encountered in the sample.

The differences observed among the number of pollen types and the Shannon-Weaver Diversity Index pertaining to samples from southern, central, and northern Greece were statistically evaluated by ANOVA. Canonical discriminant analysis (CDA) was performed on the frequency of each taxon in the samples (independent variables), in order to classify the pollen from the three different areas of 1: southern Greece, 2: central Greece, and 3: northern Greece (grouping variable). The analyses were carried out using SPSS 17.0 software for Windows (SPSS, Chicago, IL, USA).

RESULTS

The melissopalynological analysis of the samples revealed 64 families and 229 pollen types represented in total. The most frequently found families were Fabaceae, Asteraceae, and Rosaceae. Brassicaceae,

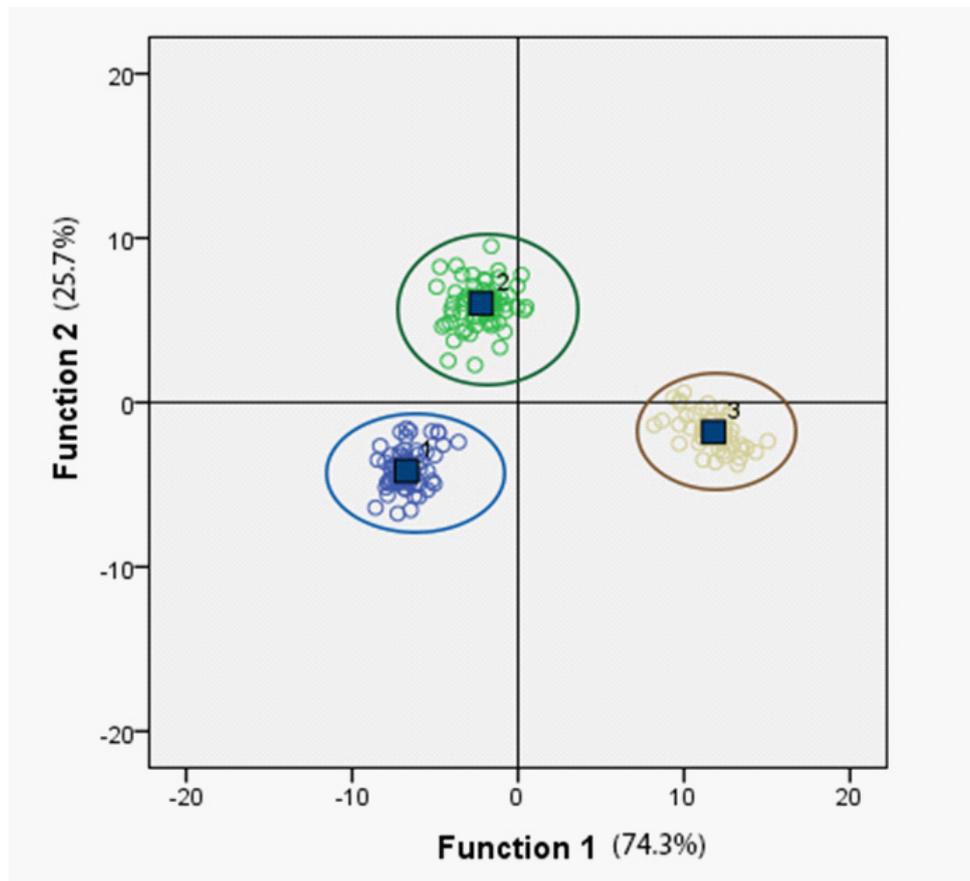


Fig. 2. Canonical Discriminant Functions among the three geographical areas in Greece (1: Southern Greece; 2: Central Greece; 3: Northern Greece).

Carduus type, *Cistus*, and *Papaver rhoeas* were found in more than half of the samples (Tab. 1). The abundance of each pollen type varied among the geographical areas and among the individual samples within a given area. The majority of the samples contained pollen from several floral sources, and about 1/3 of the samples were mainly derived from

a single botanical source (class IV).

The number of pollen types found in each sample varied from 4 to 22, and the average and standard deviation of all samples were 12.5 ± 4.0 types. The Shannon-Weaver Diversity Index values ranged from 0.2 to 3.2 in the samples from southern Greece, from 0.3 to 2.4 in the samples from central Greece,

Table 1. Pollen type frequency classes ("Class I," rare, found in <3%; "Class II," very few, found in 3-15%; "Class III," few, found in 16-45%; "Class IV," frequent, found in >45%) and number of samples (n) analyzed in northern, central, and southern Greece

Family	Pollen type	North Greece (n = 111)			Central Greece (n = 96)			South Greece (n = 78)			Greece (n = 285)		
		min	max	n	min	max	n	min	max	n	min	max	n
Actinidiaceae	<i>Actinidia chinensis</i>	I	IV	5	I	I	5	-	-	0	-	IV	10
Anacardiaceae	<i>Cotinus</i> type	I	III	2	-	-	0	-	-	0	-	III	2
	<i>Pistacia</i> ⁽²⁾	-	-	0	I	III	5	I	IV	23	IV	IV	28
	<i>Conium</i> type ⁽²⁾	I	I	1	-	-	0	I	III	2	III	III	3
	<i>Daucus</i> type ⁽³⁾	I	IV	25	I	II	23	I	II	14	IV	IV	62
	<i>Foeniculum</i> type	-	-	0	I	I	7	-	-	0	-	I	7
	<i>Pastinaca</i>	II	IV	4	I	I	9	-	-	0	-	IV	13
	<i>Scandix</i> type	-	-	0	I	I	2	I	I	2	I	I	4
Araliaceae	<i>Acanthopanax</i> type	I	II	5	-	-	0	-	-	0	-	II	5
Areaceae	<i>Chamaerops humilis</i>	I	II	2	-	-	0	-	-	0	-	II	2
Asteraceae	<i>Anthemis</i> type	I	I	1	I	II	6	I	III	13	III	III	20
	<i>Artemisia</i>	I	IV	3	-	-	0	-	-	0	-	IV	3
	<i>Calendula</i>	-	-	0	-	-	0	I	I	1	I	I	1
	<i>Carduus</i> type ⁽²⁾	I	III	60	I	IV	62	I	III	43	IV	IV	165
	<i>Carthamus</i>	I	I	5	-	-	0	I	I	3	I	I	8
	<i>Centaurea scabiosa</i>	I	IV	2	I	II	2	I	III	28	IV	IV	32
	<i>Centaurea solstitialis</i>	I	III	2	-	-	0	-	-	0	-	III	2
	<i>Cichorium</i>	I	II	5	I	I	2	II	III	4	III	III	11
	<i>Cichorium</i> type	-	-	0	-	-	0	I	I	1	I	I	1
	<i>Cirsium</i> type	I	II	11	I	III	21	I	IV	26	IV	IV	58
	<i>Gossypium hirsutum</i>	I	I	2	-	-	0	I	I	1	I	I	3
	<i>Helianthus annuus</i>	I	IV	11	II	II	1	-	-	0	IV	IV	12
	<i>Matricaria chamomilla</i>	I	II	10	I	II	10	I	IV	18	IV	IV	38
	<i>Sonchus</i> type	I	IV	42	I	III	23	I	III	11	IV	IV	76
	<i>Taraxacum officinale</i>	I	II	4	I	III	7	I	III	35	III	III	46
	<i>Tragopon</i> type	I	II	5	I	I	4	I	I	3	II	II	12

Table 1. Continued

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		min	max	n	min	max	n	min	max	n	min	max	n
Boraginaceae	<i>Borago</i>	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Cerithe</i>	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Echium</i>	-	-	0	I	I	7	I	II	4	II	II	11
	<i>Myosotis</i>	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Phacelia</i>	I	I	4	-	-	0	-	-	0	I	I	4
Brassicaceae	<i>Brassicaceae</i>	I	IV	59	I	IV	47	I	IV	50	IV	IV	156
	<i>Campanula</i>	-	-	0	-	-	0	I	I	1	I	I	1
Capparaceae	<i>Campanula type</i> ⁽²⁾	I	I	8	-	-	0	-	-	0	I	I	8
	<i>Capparis type</i>	I	II	7	-	-	0	I	II	4	II	II	11
	<i>Lonicera</i>	I	I	7	-	-	0	I	I	1	I	I	8
	<i>Sambucus</i>	I	II	4	-	-	0	-	-	0	II	II	4
	<i>Symphoricarpos type</i>	-	-	0	I	I	2	-	-	0	I	I	2
Celastraceae	<i>Viburnum type</i>	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Euonymus type</i>	-	-	0	II	III	2	-	-	0	III	III	2
	<i>Chenopodiaceae</i> ⁽³⁾	I	I	7	-	-	0	-	-	0	I	I	7
	<i>Cistus</i> ⁽²⁾	I	IV	16	I	IV	78	I	IV	54	IV	IV	148
	<i>Helianthemum</i>	I	I	2	-	-	0	-	-	0	I	I	2
Citraceae	<i>Helianthemum type</i>	-	-	0	-	-	0	I	II	2	II	II	2
	<i>Citrus</i> ⁽²⁾	-	-	0	I	III	6	I	I	2	III	III	8
	<i>Calystegia</i>	I	II	2	-	-	0	-	-	0	II	II	2
	<i>Convolvulus</i> ⁽³⁾	I	IV	22	I	IV	17	I	III	19	IV	IV	58
	<i>Corylus</i>	-	-	0	-	-	0	I	I	3	I	I	3
Cupressaceae	<i>Cupressus</i>	-	-	0	-	-	0	I	III	4	III	III	4
	<i>Curcubitaceae</i>	-	-	0	I	III	6	-	-	0	III	III	6
	<i>Citrullus type</i>	-	-	0	-	-	0	I	I	1	I	I	1
	<i>Cucumis type</i> ⁽²⁾	II	II	1	III	III	1	-	-	0	III	III	2
	<i>Ecbalium elaterium</i>	I	I	2	-	-	0	-	-	0	I	I	2

Table 1. Continued

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		min	max	n	min	max	n	min	max	n	min	max	n
Cyperaceae	<i>Carex</i>	I	I	1	-	-	0	-	-	0	I	I	1
	Cyperaceae	I	I	3	I	II	5	I	II	3	II	II	11
Dispacaceae	<i>Cephalaria</i>	I	III	15	I	II	14	I	II	14	III	III	43
Ericaceae	<i>Erica</i>	-	-	0	I	III	2	I	I	1	III	III	3
Euphorbiaceae	<i>Mercurialis</i> type	II	II	2	I	I	1	I	III	3	III	III	6
Fabaceae	<i>Acacia cyanophylla</i>	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Aesculus</i>	II	II	1	I	I	1	-	-	0	II	II	2
	<i>Amorpha fruticosa</i>	I	II	2	-	-	0	-	-	0	II	II	2
	<i>Amorpha</i> type	I	II	2	-	-	0	-	-	0	II	II	2
	<i>Anthyllis</i>	I	I	2	-	-	0	-	-	0	I	I	2
	<i>Bauhinia</i> type	-	-	0	I	I	1	-	-	0	I	I	1
	<i>Calicotome</i> type	I	I	4	I	I	2	-	-	0	I	I	6
	<i>Cercis</i> type	-	-	0	I	III	7	-	-	0	III	III	7
	<i>Ebenus</i>	I	I	1	I	I	2	I	II	7	II	II	10
	<i>Genista</i> type ⁽⁴⁾	I	III	23	I	III	10	I	III	13	III	III	46
	<i>Lotus</i>	I	I	5	-	-	0	-	-	0	I	I	5
	<i>Psoralea</i>	-	-	0	I	I	2	I	I	8	I	I	10
	<i>Trifolium</i> ⁽¹⁶⁾	I	II	27	I	IV	77	I	III	27	IV	IV	131
	<i>Trifolium repens</i>	I	III	10	I	II	12	I	II	7	III	III	29
	<i>Vicia</i> ⁽⁶⁾	I	III	21	I	II	6	I	III	3	III	III	30
Fagaceae	<i>Castanea sativa</i>	-	-	0	II	IV	14	-	-	0	IV	IV	14
	<i>Quercus</i> ⁽⁶⁾	I	III	17	I	III	11	I	IV	38	IV	IV	66
	<i>Quercus ilex</i>	I	I	2	I	III	3	-	-	0	III	III	5
	<i>Quercus</i> type	-	-	0	-	-	0	I	I	1	I	I	1
Gentianaceae	<i>Centaurium</i>	I	I	1	-	-	0	-	-	0	I	I	1

Table 1. Continued

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Family	Pollen type	North Greece (n = 111)			Central Greece (n = 96)			South Greece (n = 78)			Greece (n = 285)		
		min	max	n	min	max	n	min	max	n	min	max	n
Geraniaceae	<i>Erodium</i> ⁽²⁾	I	II	13	I	II	5	I	I	1	II	19	
	<i>Geranium</i> ⁽³⁾	I	I	1	I	II	4	I	III	13	III	18	
Hypericaceae	<i>Hypericum</i> ⁽³⁾	I	IV	24	I	III	7	II	II	2	IV	33	
	<i>Hypericum</i> type	I	II	8	I	I	4	-	-	0	II	12	
Iridaceae	Iridaceae	I	II	9	I	II	6	I	II	3	II	18	
	<i>Juglans nigra</i>	-	-	0	I	I	1	I	I	6	I	7	
Lamiaceae	<i>Ballota</i> type	II	II	1	-	-	0	-	-	0	II	1	
	<i>Mentha</i>	I	I	2	I	I	2	-	-	0	I	4	
Phlomis type	Phlomis type	-	-	0	I	I	4	I	II	11	II	15	
	<i>Salvia</i>	II	II	1	-	-	0	I	I	1	II	2	
Stachys type	Stachys type	II	II	1	-	-	0	I	I	3	II	4	
	<i>Teucrium</i>	I	I	6	-	-	0	-	-	0	I	6	
Thymus	Thymus	-	-	0	-	-	0	I	I	1	I	1	
	<i>Vitex agnus-castus</i>	I	I	1	I	I	2	-	-	0	I	3	
Lauraceae	<i>Laurus nobilis</i>	-	-	0	I	I	1	-	-	0	I	1	
	<i>Allium</i> type ⁽²⁾	I	I	2	I	I	1	I	I	3	I	6	
Liliaceae	<i>Asparagus</i>	I	I	1	-	-	0	-	-	0	I	1	
	<i>Asphodelous</i>	-	-	0	II	II	2	I	III	9	III	11	
Lythraceae	<i>Lagerstroemia indica</i>	-	-	0	I	I	1	-	-	0	I	1	
	<i>Melia azedarach</i>	-	-	0	I	I	2	-	-	0	I	2	
Myrtaceae	<i>Eucalyptus</i> ⁽²⁾	-	-	0	I	IV	4	I	II	3	IV	7	
	<i>Eleagnus</i>	II	II	1	-	-	0	-	-	0	II	1	
Oleaceae	<i>Jasminum fruticans</i>	I	I	3	-	-	0	-	-	0	I	3	
	<i>Ligustrum</i>	I	II	4	-	-	0	-	-	0	II	4	
Onagraceae	<i>Olea europaea</i>	I	IV	18	I	IV	27	I	IV	54	IV	99	
	Onagraceae	I	II	6	I	I	3	-	-	0	II	9	

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		min	max	n	min	max	n	min	max	n	min	max	n
Oxalidaceae	<i>Oxalis</i>	-	-	0	-	-	0	0	I	III	27	III	27
Papaveraceae	<i>Hypocoum</i>	I	II	5	-	-	0	-	-	0	0	II	5
	<i>Papaver rhoeas</i>	I	III	61	I	III	47	I	II	50	III	III	158
	<i>Papaver</i> type ⁽²⁾	I	III	52	I	IV	23	I	I	6	IV	IV	81
Passifloraceae	<i>Passiflora</i>	I	I	2	-	-	0	-	-	0	I	I	2
Pinaceae	<i>Pinus halepensis</i>	I	I	1	-	-	0	I	I	3	I	I	4
Plantaginaceae	<i>Veronica</i>	-	-	0	-	-	0	I	I	2	I	I	2
Platanaceae	<i>Platanus</i>	-	-	0	-	-	0	I	I	1	I	I	1
Poaceae	<i>Oryza sativa</i>	II	II	1	-	-	0	-	-	0	-	II	1
	Poaceae ⁽⁴⁾	I	II	23	I	IV	15	I	II	4	IV	IV	42
	<i>Zea mays</i>	I	II	5	I	III	6	-	-	0	III	III	11
Polygonaceae	<i>Rumex</i> ⁽²⁾	I	I	6	II	II	1	-	-	0	II	II	7
	<i>Rumex</i> type	-	-	0	I	II	2	-	-	0	II	II	2
Portulacaceae	<i>Portulaca oleraceae</i>	I	I	2	-	-	0	-	-	0	I	I	2
	<i>Portulaca</i> type	-	-	0	-	-	0	I	I	1	I	I	1
Ranunculaceae	<i>Anemone</i>	I	I	5	I	I	1	-	-	0	I	I	6
	<i>Ranunculus</i> ⁽³⁾	I	III	23	I	II	16	I	II	5	III	III	44
	<i>Thalictrum</i> type	-	-	0	I	I	1	I	II	3	II	II	4
Resedaceae	<i>Reseda</i> type ⁽²⁾	I	IV	9	I	II	4	-	-	0	IV	IV	13
Rhamnaceae	<i>Paliurus spina-christi</i>	I	II	18	I	IV	18	I	III	7	IV	IV	43
	<i>Rhamnus</i>	-	-	0	II	II	2	I	II	10	II	II	12
	<i>Rhamnus</i> type	II	III	4	I	IV	2	-	-	0	IV	IV	6

Table 1. Continued

Pollen type frequency classes ("Class I," rare, found in <3%; "Class II," very few, found in 3-15%; "Class III," few, found in 16-45%; "Class IV," frequent, found in >45%) and number of samples (n) analyzed in northern, central, and southern Greece

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		min	max	n	min	max	n	min	max	n	min	max	n
Rosaceae	<i>Cydonia japonica</i> type	-	-	0	-	-	0	I	II	5	II	5	5
	<i>Fragaria</i> type	-	-	0	-	-	0	I	I	2	I	2	2
	<i>Poterium</i> ⁽²⁾	-	-	0	I	I	1	I	III	9	III	10	10
	<i>Prunus amygdalus</i>	I	I	3	-	-	0	-	-	0	I	3	3
	<i>Prunus armeniaca</i>	I	I	1	I	I	1	I	I	2	I	4	4
	<i>Prunus avium</i>	-	-	0	II	III	2	I	I	1	III	3	3
	<i>Prunus</i> type ⁽⁵⁾	I	IV	18	I	II	9	I	III	18	IV	45	45
	<i>Robinia pseudoacacia</i>	I	III	8	I	I	1	-	-	0	III	9	9
	<i>Robinia</i> type	I	I	2	-	-	0	-	-	0	I	2	2
	<i>Rosa</i> type ⁽²⁾	I	II	6	-	-	0	-	-	0	II	6	6
Salicaceae	<i>Rubus</i>	I	IV	45	I	II	27	I	II	11	IV	83	83
	<i>Rubus</i> type ⁽³⁾	I	II	7	I	II	11	I	II	6	II	18	18
	<i>Salix</i>	I	II	6	I	IV	3	-	-	0	IV	9	9
Scrophulariaceae	<i>Verbascum</i>	I	IV	36	I	III	11	I	III	22	IV	69	69
	<i>Verbascum</i> type ⁽²⁾	I	IV	12	I	II	9	I	IV	10	IV	31	31
	<i>Veronica</i>	-	-	0	I	IV	6	I	II	7	IV	13	13
Smilacaceae	<i>Smilax</i>	I	II	2	I	III	2	-	-	0	III	4	4
	<i>Tamarix</i> type	I	I	2	-	-	0	-	-	0	I	2	2
Thymaleaceae	<i>Thymalea</i>	-	-	0	-	-	0	I	I	2	I	2	2
	<i>Daphne</i>	-	-	0	-	-	0	I	II	2	II	2	2
Tiliaceae	<i>Tilia</i>	I	I	6	I	I	4	-	-	0	I	10	10
	<i>Ulmus</i> type	-	-	0	I	III	3	-	-	0	III	3	3

Table 1. Continued
 Pollen type frequency classes ("Class I," rare, found in <3%; "Class II," very few, found in 3-15%; "Class III," few, found in 16-45%; "Class IV," frequent, found in >45%) and number of samples (n) analyzed in northern, central, and southern Greece

Family	Pollen type	North Greece (n = 111)			Central Greece (n = 96)			South Greece (n = 78)			Greece (n = 285)		
		min	max	n	min	max	n	min	max	n	min	max	n
Urticaceae	<i>Urtica</i> (2)	I	I	1	I	I	2	I	I	2	I	I	5
Verbenaceae	<i>Verbena</i>	-	-	0	II	II	1	-	-	0	II	II	1
Violaceae	<i>Viola</i>	-	-	0	-	-	0	I	II	5	II	II	5
Vitaceae	<i>Parthenocissus</i>	I	IV	16	I	II	6	II	II	3	IV	IV	25
	<i>Vitis vinifera</i>	I	III	4	I	I	1	I	I	1	III	III	6
Xanthorrhoeaceae	<i>Asphodelous</i>	I	I	1	-	-	0	I	I	1	I	I	2
Zygophyllaceae	<i>Tribulus terrestris</i>	I	I	3	-	-	0	-	-	0	-	-	3
-	Tricolporate pollen form (6)	I	IV	17	I	I	2	I	III	13	IV	IV	32

The number in parentheses next to the pollen type indicates the number of taxa found, reflecting different color of pollen loads and/or morphological variation of pollen grains.

and from 0.3 to 2.1 in the samples from northern Greece. On average, the Shannon-Weaver Diversity Index value of all samples was 2.1 ± 0.3 . ANOVA analysis showed a statistically significant difference among the three geographical areas considering the number of pollen types ($p < 0.001$) and the Shannon-Weaver Diversity Index values ($p < 0.001$). In particular, samples from southern Greece showed statistically significantly more pollen types and higher Shannon-Weaver Diversity Index values than those from northern and central Greece. In total, 141 pollen types were identified in the samples collected in northern Greece. Brassicaceae, *Carduus* type, *Sonchus* type, *Rubus*, *Papaver rhoeas*, and *Papaver* type were found in a great number of samples from this area (Tab. 1). Pollen loads from *Actinidia chinensis*, *Artemisia*, Brassicaceae, *Cistus*, *Convolvulus*, *Centaurea scabiosa*, *Daucus* type, *Helianthus annuus*, *Hypericum*, *Olea europaea*, *Parthenocissus*, *Pastinaca*, *Prunus* type, *Reseda* type, *Rubus*, *Sonchus* type, *Verbascum*, and *Verbascum* type were observed as class IV components (greater than 45%) in samples from this locale (Tab. 1). These samples exhibited wide pollen diversity; only Brassicaceae pollen was represented in several samples (14). Brassicaceae, *Carduus* type, *Cistus*, *Papaver rhoeas*, and *Trifolium* were found in several samples from central Greece (Tab. 1). In total, 132 taxa were identified. In 64 out of 96 samples from this area, the botanical origin of the pollen loads was mainly from a single source (class IV); and the majority of these comprised pollen from Brassicaceae and *Cistus*. In southern Greece, 108 pollen types were identified. Brassicaceae, *Cistus*, *Olea europaea*, and *Papaver rhoeas* were detected in over 60% of the samples. Asteraceae (*Carduus* type, *Cirsium* type, *Centaurea scabiosa*, and *Taraxacum officinale*) were also identified in several cases (Tab. 1). In about 35% of the samples from southern Greece the collected pollen was defined as class IV, mainly from *Cistus* plants. Statistical analysis (CDA) showed complete separation among the three geographical areas sampled (Fig. 2). According to the classifications results, 100% of the original grouped cases and 75% of the cross-validated grouped cases were classified correctly. The statistical analysis revealed two functions. The first discriminates the pollen from northern Greece from the other two sources, especially from southern Greece; and the second discriminates the pollen from central Greece from the samples from other geographical origins, particularly from the pollen from southern Greece.

DISCUSSION

Pollen provides the sole protein source for the honey bee colonies, and it is necessary for brood and young worker bee development. Therefore, colony survival and growth depends on both the quantity and the nutritional quality of the pollen provided by the foragers in the colony. The composition of pollen harvested by honey bees can vary greatly depending on the time of year, the botanical origin, and the geographic region from which the pollen is obtained (Linskens and Jorde, 1997).

The determination of the botanical and geographical origin is fundamental in understanding pollen quality. Both parameters influence its nutrient value. They affect not only the development and survival of the honey bee colony, but also the value of the pollen as a commercialized product marketed for human consumption. Consequently, the local flora directly and indirectly affects beekeepers' profits by affecting the productivity of the honey bee colony as well as the price of the product in the marketplace.

Many authors have studied the botanical origin of pollen collected from honey bees in diverse geographical areas. Freitas et al. (2013) collected samples of pollen loads from honey bee colonies within 19 municipalities in Brazil and analyzed their sediment. They found 4 to 25 pollen types per macro region. The study conducted by Novais et al. (2010) on trapped pollen from *Apis mellifera* colonies in the Caatinga region of Brazil revealed 85 pollen types in samples collected within a two year period. Andrada and Telleria (2005) analyzed honey bee pollen loads in the Calden district of Argentina and recorded 29 pollen sources over two apicultural periods. Forcone et al. (2011; 2013) collected bee pollen from the Andean region of Chubut and the region northwest of Santa Cruz (Argentinean Patagonia) and found 46 and 42 pollen types, respectively, during one apicultural season, with over 16 taxa represented per sample in some cases. Kajobe (2007) studied pollen foraging by *Apis mellifera* in Bwindi Impenetrable National Park (Uganda) for one year and found 71 pollen sources in total, and a Shannon-Weaver Diversity Index value of 1.5. Wróblewska et al. (2010), in an 8-year-study of beebread in four Lublin regions in Poland, found 40, 57, 58 and 73 pollen types, respectively, in the samples collected. Dimou and Thrasyvoulou (2007a) found over 140 pollen taxa in Thessaloniki, Greece, in a two year study. Bilisik et al. (2008) recorded 41 pollen types in the Bursa region of Turkey across one apicultural period and found on average about 8 taxa per sample. During the same period, Avni et al. (2009) reported

that the number of honey bee pollen sources in four distinct sites in Israel ranged from 5 to 20 taxa per sample.

However, because of the different methodologies followed in these studies (i.e., variation in time and duration of sampling, method of pollen analysis, presentation of data) it is hard to compare them to one another. In this study, the average number of pollen types detected per sample as well as the high Shannon-Weaver Diversity Index value indicates a great variety of pollen sources in the collected samples. This result is consistent with the species richness characteristic of the vegetation in Greece (Phitos et al., 1995). Pollen from different plant species has different nutrient content. Therefore, having variety and diversity of pollen types is desirable since it results in higher nutritional value within the final product. In this study, most collected samples showed the presence of Brassicaceae, *Carduus* type, and *Papaver rhoeas* pollen, which has been previously shown to have high protein content (>20%), a characteristic that not only satisfies colony requirements for development and optimum production (Herbert, 1992; Szczęśna, 2006; Liolios et al., 2010) but can also result in a rise in the crude total protein content of the commercial product.

Finally, despite the extended bibliography on pollen foraging behavior, there is no data about possible discrimination among different geographical areas by honey bees. The results of this study enable such research by showing that the determination of the geographical origin of the honey bee-collected pollen is possible, even over areas that are relatively close to each other. Samples from northern, central, and southern Greece were clearly distinguished from each other. This observation may also support establishment of a Protected Geographical Indication or Protected Designation of Origin of the commercial product.

CONCLUSIONS

Understanding the composition and diversity of the local pollen flora is very important to the beekeeping industry of the region, both in terms of colony health and in the value of bee pollen as a commercial product. In recent years, labeling of the botanical and geographical origin of products is an increasing requirement for all foods marketed for human consumption, which is directly connected to quality control and to the consumer's perception of the product and thus its commercial value.

In this study we show that (i) Greek pollen is characterized by a great variety of pollen sources; and (ii) determination of the geographical origin of bee pollen is possible even across small-scale areas.

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