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DYNAMICS OF SHORT-TERM VARIATION IN POLLEN FORAGING BY HONEY BEES

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A pollen trap was placed at the entrance of two bee colonies on 10 days during a 25 days period in Lazarim, 10 km south of Lisbon, Portugal. On each day, pollen samples were collected from 9.30 to 11.30 h (morning sample) and 11.30 to 13.30 h (midday sample). A total of 49 pollen types were identified in 31 pollen samples, but only the 20 most abundant pollen types were included in the ordination analysis with the software package, CANOCO. There was significant difference between the number of pollen types encountered in morning samples and midday samples. Abundance of each pollen type per sample was transformed into proportional pollen volume. Three significant trends were outlined in the ordination analysis of the qualitative composition of pollen samples: (1) a gradual change in the importance of pollen types over time, (2) a significant difference between morning and midday samples, (3) a significant difference between colonies. Ambient temperature, relative air humidity, size of the pollen sample, and number of pollen types in the pollen samples did not explain a significant part of the total variance among pollen samples. The conducted ordination analysis allowed us to outline temporal patterns in the pollen foraging behavior on colony level.

Key words: *Apis mellifera*, ordination, pollen foraging, pollen analysis, Portugal.

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Uma armadilha para captura de pólen foi colocada à entrada de duas colónias de abelhas do mel, durante um período de 25

dias, em Lazarim, localidade situada a 10 km a sul de Lisboa, Portugal. Procedeu-se à recolha de amostras de pólen todos os dias, entre as 9.30 e as 11.30 h (amostra da manhã) e entre as 11.30 e as 13.30 h (amostra do meio dia). Foram identificados 49 tipos de pólen em 31 amostras, mas só os 20 tipos mais abundantes foram considerados na análise de ordenação usando o programa CANOCO. Existe uma diferença significativa entre o número de tipos de pólen encontrados nas amostras da manhã e nas amostras do meio dia. A abundância de cada tipo de pólen por amostra foi transformada no proporcional volume de pólen. Foram encontradas três tendências significativas pela análise de ordenação da composição qualitativa das amostras de pólen: (1) uma mudança gradual na importância dos tipos de pólen ao longo do tempo, (2) uma diferença significativa entre a composição das amostras da manhã e das amostras do meio dia, (3) uma diferença significativa entre as colónias. A temperatura ambiente, a humidade relativa do ar, o tamanho das amostras de pólen e o número de diferentes tipos de pólen nas amostras, não explica parte significativa da variância total encontrada entre as amostras de pólen. A análise de ordenação efectuada permitiu-nos encontrar padrões temporais no comportamento na recolha do pólen a nível da colónia.

Palavras chave: *Apis mellifera*, ordenação, recolha de pólen, análise do pólen, Portugal.

INTRODUCTION

According to STANLEY & LINSKENS (1974), pollen is the ultimate protein and lipid source to larvae and imagines of Apidae species, and a honey bee larva requires about 145 mg pollen to complete its instar phases. Comparatively, adult honey bee pollen foragers only consume small amounts of pollen (CAMAZINE 1993). CAMAZINE (1993) showed that pollen foraging of individual honey bees changed significantly from one day to the next, when the pollen supplies in the hives were manipulated. From additional experiments, he concluded that the nurse bees play a major role in the regulation of pollen foraging on colony-level. It is therefore not surprising that size of bee colonies (BEAUCHAMP 1992, ECKERT *et al.* 1994) and amount of brood in the hive (FREE 1967, HELLMICH & ROTHENBUHLER 1986, FEWELL & WINSTON 1996, CAMAZINE *et al.* 1998) have been found to affect the relative pollen foraging effort on colony level. Quantitative studies of pollen foraging have also shown that the pollen foraging effort of a bee colony can be modified by directional genetic selection of bee strains (GUZMAN-NOVOA & GARY 1993, PAGE *et al.* 1995) and varies between species of stingless bees (BRUIJN & SOMMEIJER 1997).

Pheromones emitted by nurse bees (CAMAZINE 1993) or the queen (HIGO *et al.* 1992) are believed to control the number of individuals devoted to pollen foraging according to the pollen requirements in the hive and the size of individual pollen loads (HIGO *et al.* 1992), but the actual pollen foraging is regulated by the pollen presentation patterns of pollen sources. Hourly pollen presentation in plants follows species specific patterns and involves mechanisms for optimising pollen exchange (VOGEL 1983). Many flowers are known to restrict the pollen presentation periods within the blooming period (e.g. SYNGE 1947, PERCIVAL 1947, 1950, 1955, FREE 1967, ABROL & BHAT 1987, GIURFA & NÚÑEZ 1992, GOODWIN & PERRY 1992, THOMSON & THOMSON 1992). Association of hourly pollen presentation and pollen foraging effort of individual honey bees has been demonstrated from flower observations (PERCIVAL 1950, 1955, THOMSON & THOMSON 1992, NANSEN & KORIE 2001). To our knowledge, there are no published studies of qualitative and quantitative changes in pollen samples collected at bee hive entrances. Evaluation of pollen foraging on colony level within and between days during a short time period may indicate how sensitive bee colonies are to subtle changes in plant phenology.

In the present study, we identified pollen types in pollen samples from two adjacent bee hives of similar size. We used the ordination software package, CANOCO (TER BRAAK, 1992), to characterise the pollen types according to their relative importance within and between days and to outline temporal trends in the pollen foraging behavior. The importance of diversifying the pollen foraging strategy on colony level is discussed.

MATERIALS AND METHODS

Experimental design

The study was conducted on 10 days from 24 March to 15 April 1995 in Lazarim, 10 km south of Lisbon, Portugal. Observations were only conducted on days with sunny weather and little or no wind. Two bee colonies of about 30,000 honey bees from the same apiary were used for the experiment in a semi-urban area dominated by *Cistus* spp., *Eucalyptus* spp., and annual grassland species. Pollen was captured with a pollen trap, as described by SYNGE (1947), during two time periods; morning (9.30-11.30 h) and midday (11.30-13.30 h). Temperature and relative air humidity were measured every 30 min with a thermohygrograph placed 30 cm above the ground next to the bee hives and the mean Temperature and relative air humidity were determined for each pollen sampling period.

Pollen identification and quantification

Pollen collected from each sampling period was dried for 24 h at 70ºC to obtain total pollen dry weight. Entire samples were diluted and homogenised in acetic acid and sub samples of 0.5 gram from each pollen sample were prepared. As recommended by MOORE *et al.* (1991), pollen samples were subjected to

acetolysis before identification, and pollen slides were mounted in silicone oil and identified under light microscope (× 400 mag. and × 1000 mag.). From each sample, 500 to 1400 pollen grains from at least two different slides were identified. Pollen identification was based on specific morphological identification keys for the different plant families. These keys were also used in combination with the pollen reference collection at the Botanical Museum of Lisbon, Portugal. As recommended by SILVEIRA (1991) and BIESMEIJER *et al.* (1992), counts of each pollen type were multiplied with an approximate estimate of pollen grain volume (measured to nearest μm under the microscope). The volume was calculated assuming a perfect geometrical shape; spherical, ellipsoid, or triangular (O´ROURKE & BUCHMANN 1991). For comparison of pollen samples, the volume of each pollen type was converted to a percentage volume out of the total volume of pollen grains counted from the sample. Genstat 3.2 for Windows was used to conduct paired t-tests for testing the difference in means of explanatory variables and number of pollen types in morning and midday samples.

Ordination

The software package, CANOCO for Windows version 4.02 (TER BRAAK 1992), was used for the ordination analyses, which are further described by JONGMAN *et al.* (1995). In ecological research, where many response variables change simultaneously, ordination techniques help to visualise relationships and gradients in a low-dimensional space by expressing the similarity in distance between points (JONGMAN *et al.* 1995). Secondly, the unit of axes is in standard deviations and therefore expresses the total amount of variance in the data set (in this case pollen types in samples within and between days). Starting with an indirect ordination technique, JONGMAN *et al.* (1995) recommended a detrended correspondence analysis, which is based on a unimodal model approximated by a gaussian curve, as the most appropriate procedure to expose underlying associations in a data set. The initial detrended correspondence analysis was used to chose the appropriate "direct" ordination analysis for testing explanatory variables: if the length of the principal axes, DCA1 and DCA2, does not exceed four standard deviations the response curves may be considered linear and a redundancy analysis is used; otherwise a canonical correspondence analysis is the most appropriate “direct” ordination technique.

After a detrended correspondence analysis, the following explanatory variables were tested in a canonical correspondence analysis: (1) a dichotomous variable coding for colony one (one) or colony two (two), (2) mean temperature (^{o}C), and (3) mean relative air humidity (%) during pollen trapping, (4) a time variable, 'Date' denoting the number of days after the first sampling date, (5) a dichotomous variable, 'Midday', coding for morning (one) or midday (two) samples, (6) number of pollen types, and (7) amount of pollen collected by the trap at the bee hive entrance. Partial canonical correspondence analysis was

conducted to examine the level of co-variance between explanatory variables. This is done by testing the significance of one explanatory variable after having removed the effect of another explanatory variable.

RESULTS

It was significantly cooler in the morning than at midday (d.f. = 9; $t = 8.62$; $P < 0.001$), and the relative air humidity was significantly higher in morning than at midday (d.f. = 9; $t = 6.87$; $P < 0.001$) (Tab. 1). The amount of pollen captured during morning and midday time periods was significantly different (*d.f.* = 9; $t = 1.79$; $P < 0.001$) in colony one, but there was no significant difference in colony two (*d.f.* = 4; $t = 0.89$; $P < 0.429$). A pairwise comparison of the number of pollen types in morning samples (mean 18.0 ± 4.6 s.d. ($n = 15$)) and midday samples (mean 21.7± 5.1 s.d. ($n = 15$)) showed that morning samples contained significantly less pollen types (*d.f.* = 14; $t = 5.14$; $P < 0.001$). Although there were significantly less pollen types in morning samples, there was a positive correlation between number of pollen types in morning and midday samples from the same day (Fig. 1) (*d.f.* = 14; $F = 30.8$; $P < 0.001$). Fig. 1 also showed that less pollen types were found in samples from colony two than from colony one, but this was probably related to the lower number of identified pollen from samples collected at colony two (see Tab. 1).

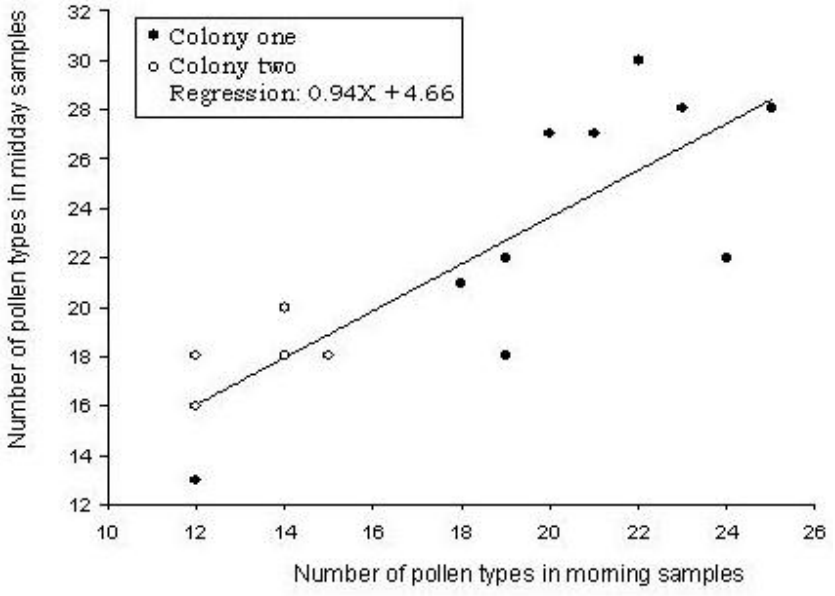

Figure 1. Correlation between number of pollen types encountered in morning (9.30-11.30) and midday (11.30-13.30) pollen samples from two bee hives (colony one: n = 10; colony two: n = 5).

Table 1. Mean temperature (°C) and relative air humidity (%) and during the morning (9.30-11.30) and midday (11.30-13.30) pollen trapping periods. Dry weight of pollen samples (in grams) collected at the bee hive entrance at the two bee hives. 'Pollen (no.)' denoted the number of pollen grains identified in the pollen sample; 'Pollen types' denoted the number of identified pollen types in each pollen sample (see Table 2). The five variables were used in a canonical correspondence analysis together with two time variables and a variable coding for the two bee hives to explain the variance in the 31 pollen samples (Fig. 3).

			Colony one			**Colony Two**		
Date	Temp. (°C)	Rel. Hum. (%)	Pollen coll. (grams)	Pollen counted (no.)	Pollen types	Pollen coll. (grams)	Pollen counted (no.)	Pollen types
Morning								
24-mar	19.4	61.0	0.4	605.6	12.0			
27-mar	20.4	52.0	1.5	1330.0	20.0			
29-mar	18.8	58.0	1.8	1320.0	22.0			
01-apr	16.6	46.0	0.4	1243.0	25.0			
03-apr	18.0	62.0	1.9	1344.0	21.0			
07-apr	18.4	78.0	2.3	1273.0	23.0	1.0	537	12
08-apr	20.2	61.0	5.3	1336.0	24.0	3.3	553	12
11-apr	21.2	66.0	2.5	1258.0	18.0	4.3	529	14
13-apr	20.2	72.0	3.8	949.0	19.0	4.8	537	15
15-apr	20.0	45.0	2.4	1257.0	19.0	7.1	538	14
Midday								
24-mar	21.4	48.0	1.7	509.6	13.0			
27-mar	22.6	48.0	3.4	1386.0	27.0			
29-mar	22.8	48.0	3.2	1502.0	30.0			
01-apr	22.0	29.0	1.5	1181.0	28.0			
03-apr	21.6	53.0	3.9	1402.0	27.0	0.7	588	17
07-apr	21.6	63.0	4.9	1480.0	28.0	0.9	632	18
08-apr	25.4	44.0	7.2	1219.0	22.0	5.5	592	16
11-apr	25.4	48.0	4.3	1384.0	21.0	6.4	556	20
13-apr	21.8	69.0	8.1	1290.0	22.0	9.1	533	18
15-apr	23.8	35.0	3.2	1243.0	18.0	4.0	510	18

A total of 49 pollen types were identified representing by identity or resemblage 29 families and 46 genera; one pollen type could not be identified (Table 2). In Table 2 the pollen types are organised according to their volumetric importance in pollen samples. Considering the 20 most abundant pollen types in morning samples from the two bee colonies, five pollen types (*Cistus, albidus, C. populifolius, Pinus, Pistacia, Plantago coronopus* type) were only identified in samples from in colony one. Considering the 20 most abundant pollen types in midday samples from the two bee colonies, two pollen types (*Cistus populifolius*

Table 2. Identified pollen types in the 31 pollen samples from either 'mor' (9.30-11.30) or 'mid' (11.30-13.30) collected at the two bee hives on 10 sampling days. 'Pollen counted' showed the total number of pollen grains identified for each pollen type; 'Found in samples' showed the number of pollen samples in which the pollen type was identified. 'No. (%)' denoted the numeric importance of the pollen type in percentage of the total number of identified pollen grains in the pollen sample; 'vol.(%)' denoted the volumetric importance of the pollen type in percentage of the total volume of identified pollen grains in the pollen sample. 'Pollen types not included in the ordination analysis' were, based on a initial detrended correspondence analysis, found to have only negligible influence on the ordination of pollen samples.

Family	**Pollen type**	**Period**	**Total pollen counted**	**Total found in samples**	**Colony one**		**Colony two**	
					No.(%)	**Vol.(%)**	**No.(%)**	**Vol.(%)**
Cistaceae	*Cistus salvifolius*	mor	1256	15	5,05	51,077	1,846	24,292
		mid	1114	16	4,362	45,103	3,416	32,793
Papaveraceae	*Papaver rhoeas* type	mor	3777	13	7,436	16,478	28,286	66,799
		mid	1273	14	2,012	5,377	11,774	21,199
Brassicaceae	*Brassicaceae*	mor	818	15	3,094	6,122	0,876	2,025
		mid	3149	16	10,063	22,132	11,634	18,463
Rutaceae	*Citrus*	mor	467	15	2,09	6,227	0,298	1,212
		mid	288	16	1,112	3,756	1,19	3,174
Unknown	*Unknown*	mor	905	15	3,774	7,134	0,389	0,913
		mid	449	15	1,453	3,203	1,691	2,865
Asteraceae	*Carduus* type	mor	134	12	0,483	3,277	0,295	1,941
		mid	136	13	0,425	3,663	0,512	2,674
Asteraceae	*Inula* type	mor	347	14	1,106	1,09	1,173	1,347
		mid	699	15	1,859	2,356	3,728	3,18
Pinaceae	*Pinus*	mor	10	6	0,037	0,924	0	0
		mid	13	11	0,026	0,77	0,092	2,244
Asteraceae	*Anthemis* type	mor	15	9	0,027	0,057	0,122	0,269
		mid	321	15	0,333	0,913	3,696	7,007
Cistaceae	*Cistus ladanifer* type	mor	47	9	0,262	2,02	0,016	0,238
		mid	54	11	0,343	2,475	0,044	0,333
Oleaceae	*Olea*	mor	109	13	0,368	0,408	0,211	0,251
		mid	464	15	1,35	2,03	1,988	1,78
Malvaceae	*Malva* type	mor	0	0	0	0	0	0
		mid	5	3	0,014	2,197	0,014	1,568
Rutaceae	*Eucalyptus*	mor	4348	15	16,665	1,154	3,11	0,225
		mid	4594	16	16,503	1,368	9,77	0,43
Cistaceae	*Cistus albidus* type	mor	5	4	0,025	0,269	0	0
		mid	21	4	0,056	0,736	0,17	1,055

Table 2 (cont.)

Family	Pollen type	Period	Total pollen counted	Total found in samples	Colony one		Colony two	
					No.(%)	Vol.(%)	No.(%)	Vol.(%)
Cistaceae	*Cistus albidus* type	mor	5	4	0,025	0,269	0	0
		mid	21	4	0,056	0,736	0,17	1,055
Plantaginacaae	*Plantago coronopus* type	mor	42	5	0,171	0,716	0	0
		mid	13	7	0,041	0,177	0,043	0,094
Boraginaceae	*Echium*	mor	1593	15	5,777	0,345	3,138	0,228
		mid	1324	16	4,156	0,303	4,706	0,26
Leguminosae	*Astragalus lusitanica*	mor	165	9	0,769	0,071	0,268	0,04
		mid	1084	15	3,587	0,523	1,703	0,199
Anachardiaceae	*Pistacia*	mor	2	2	0,007	0,018	0	0
		mid	76	9	0,336	0,637	0	0
Caryophyllaceae	*Spergula* type	mor	15	5	0,048	0,069	0,03	0,035
		mid	73	7	0,205	0,289	0,375	0,285
Cistaceae	*Cistus populifolius*	mor	1	1	0,004	0,044	0	0
		mid	7	3	0,027	0,445	0	0
Pollen types not included in ordination analysis								
Resedaceae	*Reseda media* type	mor	104	5	0,414	0,077	0,031	0,006
		mid	162	8	0,577	0,134	0,3	0,041
Rosaceae	*Rubus* type	mor	85	9	0,259	0,043	0,281	0,045
		mid	217	16	0,702	0,148	0,823	0,113
Rosaceae	*Crataegus* type	mor	85	9	0,325	0,199	0	0
		mid	92	8	0,346	0,231	0,029	0,017
Umbellifera	*Chaerophyllum hirsutum*	mor	27	5	0,073	0,007	0,126	0,014
		mid	95	9	0,227	0,036	0,609	0,058
Leguminosae	*Trifolium* type	mor	45	7	0,144	0,082	0,1	0,059
		mid	30	5	0,112	0,063	0	0
Asteraceae	*Liguliflora*	mor	42	8	0,244	0,33	0,015	0,037
		mid	8	6	0,027	0,074	0,015	0,031
Rosaceae	*Potentilla* type	mor	16	6	0,03	0,005	0,142	0,025
		mid	52	5	0,022	0,006	0,719	0,113
Liliaceae	*Liliaceae*	mor	17	4	0,068	0,017	0	0
		mid	24	4	0,097	0,043	0	0
Crassulaceae	*Sedum*	mor	4	2	0,015	0,002	0	0
		mid	49	7	0,169	0,036	0,132	0,014
Rosaceae	*Geum* type	mor	0	0	0	0	0	0
		mid	41	4	0,167	0,043	0	0

Table 2 (cont.)

Family	Pollen type	Period	Total pollen counted	Total found in samples	Colony one		Colony two	
					No.(%)	Vol.(%)	No.(%)	Vol.(%)
Poaceae	*Poaceae*	mor	0	0	0	0	0	0
		mid	21	3	0,078	0,103	0	0
Leguminosae	*Ononis* type	mor	2	1	0,008	0,001	0	0
		mid	12	2	0,103	0,003	0	0
Leguminosae	*Ulex* type	mor	9	4	0,036	0,003	0	0
		mid	1	1	0,004	0,001	0	0
Labiatae	*Labiatae*	mor	6	3	0,022	0,049	0	0
		mid	0	0	0	0	0	0
Caryophyllaceae	*Silene* type	mor	0	0	0	0	0	0
		mid	5	3	0,018	0,232	0	0
Oxalidaceae	*Oxalis articulata* type	mor	1	1	0,004	0,044	0	0
		mid	4	4	0,015	0,223	0	0
Labiatae	*Lavandula* type	mor	2	1	0,008	0,008	0	0
		mid	3	3	0,011	0,015	0	0
Polygonaceae	*Rumex acetosella* type	mor	3	3	0,012	0,024	0	0
		mid	2	2	0,007	0,014	0	0
Ranunculaceae	*Ranunculaceae*	mor	5	2	0,019	0,073	0	0
		mid	0	0	0	0	0	0
Geraniaceae	*Erodium*	mor	2	2	0,007	0,091	0	0
		mid	0	0	0	0	0	0
Plumbaginaceae	*Armeria* type	mor	0	0	0	0	0	0
		mid	1	1	0,004	0,103	0	0
Leguminosae	*Acacia*	mor	1	1	0,004	0,047	0	0
		mid	0	0	0	0	0	0
Labiatae	*Salvia*	mor	1	1	0,004	0,028	0	0
		mid	0	0	0	0	0	0
Schrophulariceae	*Schrophularia* type	mor	0	0	0	0	0	0
		mid	2	1	0,007	0,017	0	0
Fagaceae	*Querus coccifera* type	mor	1	1	0,004	0,006	0	0
		mid	1	1	0,004	0,004	0	0
Umbellifera	*Oenanthe fistulosa*	mor	0	0	0	0	0	0
		mid	1	1	0,004	0	0	0
Asteraceae	*Senecio* type	mor	1	1	0,004	0,007	0	0
		mid	0	0	0	0	0	0
Aizoaceae	*Carpobrotus* type	mor	0	0	0	0	0	0
		mid	1	1	0,004	0,005	0	0

and *Pistacia*) were only identified in samples from in colony one. None of the 20 most abundant pollen types were only found in samples from colony two. Five pollen types (Brassicaceae, *Cistus salvifolius, Citrus, Eucalyptus* and *Echium*) were encountered in all 31 samples, while most of the pollen types were found in less than a third of the samples. Transformation of numerical importance into pollen volume meant that the importance of small pollen, like *Eucalyptus* and *Echium*, declined, while the importance of large pollen (e.g. *Cistus salvifolius*, Brassicaceae, *Malva* type, Pinus, and *Papaver rhoeas* type) increased.

Ordination of the pollen spectrum

Due to the many pollen types involved it is difficult to visualise clear patterns, but ordination techniques use simple mathematical procedures to outline underlying trends in a two dimensional space. An initial detrended correspondence analysis (not shown) including all 49 pollen samples showed that 47.4% of the total variance was explained by the two principal axes, DCA1 and DCA2. A second detrended correspondence analysis was conducted (Fig. 2) in which only the 20 most abundant pollen types were included, and almost the same amount of variation could be explained by the principal ordination axis (50.2%). Omitting the 29 most rare pollen types had therefore negligible effect on the ordination analysis of pollen samples. In Fig 2, midday pollen samples were mainly located along the positive side of the second axis, DCA2, and morning samples along the negative side. Hence, DCA2 denoted the difference between morning and midday samples. Morning samples from the two colonies were distinctly separated along the positive side of the principal axis, DCA1, while the midday samples from the two colonies were only loosely separated. The analysis suggested therefore that the two bee colonies mainly foraged different pollen types in the morning time period. In the canonical correspondence analysis (Fig. 3), the tested explanatory variables were selected individually by order of importance, and the temporal change in pollen samples ('Days') explained most of the total variation ($F = 6.615$; $P = 0.005$). This means that the qualitative composition of pollen types changed significantly within the trapping period of 25 days. The difference between morning and midday samples ('Midday')was the second most important explanatory variable ($F = 6.480$; $P = 0.005$), which means that the relative importance of the pollen types was significantly different between morning and midday samples. Thirdly, the difference between colonies ('Hive') was found to be significant ($F = 5.311$; $P = 0.005$). The remaining explanatory variables: amount of pollen collected in the trap, number of pollen types in samples, temperature, and relative humidity could not explain a significant part of the variance among samples ($P > 0.05$). The significant difference between the two hives could be attributed to the less pollen grains identified in samples from colony two compared to colony one. Therefore, a partial canonical correspondence analysis was conducted where, the difference between bee hives was examined after having removed the effect of

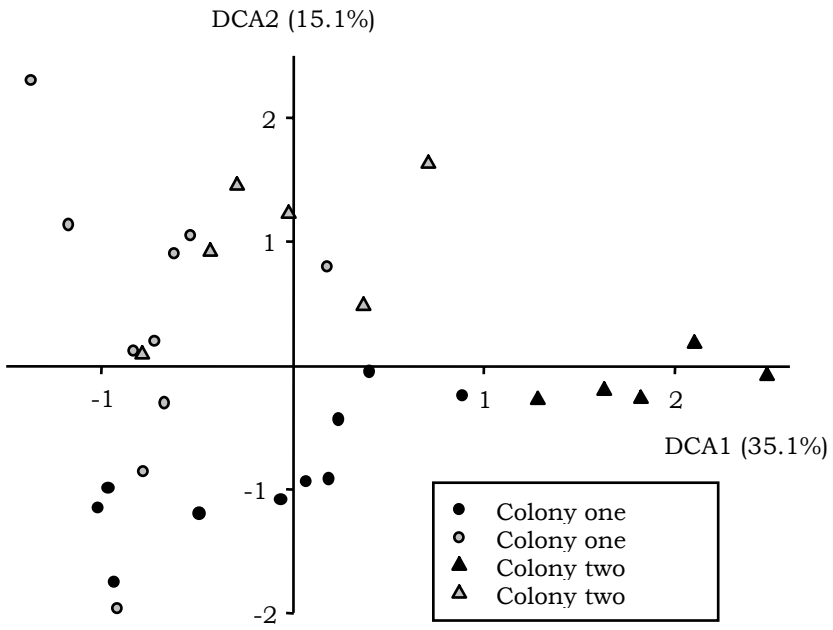

Figure 2. Detrended correspondence analysis of the 20 most important pollen types in the 31 pollen samples from the morning (9.30-11.30) or midday (11.30-13.30) from two bee hives. Axes are in standard deviation units. Percentages show the amount of variance explained by the principal axes.

the number of identified pollen grains (Table 1), but the difference between bee hives remained significant (F = 5.653; P = 0.005). Due to the apparently close correlation between the explanatory variables, 'Hive' and 'Days', in Fig. 3 it was not known whether the significant difference between the two colonies was associated with the temporal trend in the importance of pollen types. Thus, a second partial canonical correspondence analysis was conducted where the effect of the two time variables, 'Days' and 'Time' was removed, and the difference between the two colonies remained significant (F = 5.30; P = 0.01). Hence, the significant difference between the two colonies was independent of the temporal variation in pollen foraging and must be related to difference in relative importance of pollen types and thereby in their choice of pollen sources. Fig. 3 showed the association of the pollen types with the significant explanatory variables from the canonical correspondence analysis. Considering the most abundant pollen types, it was seen that *Cistus salvifolius* was not associated with any of the time variables and therefore located near the centre of Fig. 3. This means that it was equally important within and between days from the two bee hives through out the trapping period. *Papaver rhoeas* type was mainly

encountered in pollen samples from the end of the study period and especially important in samples from colony two. Brassicaceae pollen were especially dominant in midday samples from both bee hives. The Unknown pollen type and *Citrus* were found mostly dominant in pollen samples from colony one from the beginning of the study period.

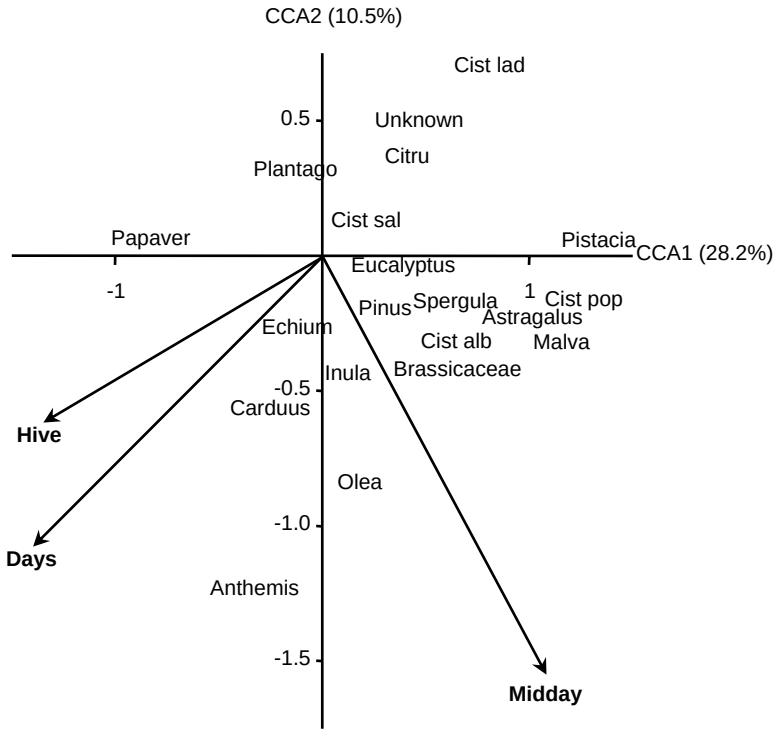

Figure 3. Canonical correspondence analysis of the 20 most important pollen types in the 31 pollen samples from the morning (9.30-11.30) or midday (11.30-13.30) from two bee hives. Axes are in standard deviation units. Percentages show the amount of variance explained by the principal axes. The three variables which explained a significant part of the total variance are shown: 'Date' denoted the direction of the change in pollen spectrum through the sampling dates; 'Midday' denoted the direction of change from morning to midday; 'Hive' denoted the significant difference between colony one and two. Axes are in standard deviation units. Percentages show the amount of variance explained by the axes.

DISCUSSION

BIESMEIJER *et al.* (1992), ORTIZ (1994) and STIMEC *et al.* (1997) analysed pollen collected at the bee hive entrance to outline seasonal changes in qualitative composition of pollen samples. Gradual seasonal changes in long

term monitoring of pollen foraging are not surprising as they probably reflect the blooming period of food plants, but much less is known about the changes in pollen foraging of bee colonies during the day and how the pollen foraging pattern on colony level coincides with pollen presentation periods in the food sources. TODD & BISHOP (1940) argued that pollen collected with traps at the bee hive entrance may not be representative for the amount of collected pollen by the colony, and SYNGE (1947), LEVIN & LOPER (1984) and GOODWIN & PERRY (1992) discussed the selectivity of pollen trapping caused by the variation in size of pollen pellets collected in different plants. LAERE & MATENS (1971), PERCIVAL (1947) and LEVIN & LOPER (1984) showed that bee colonies compensated for pollen losses due to a pollen trap at the bee hive entrance, which means that the pollen trap influences on the foraging behavior. For these reasons interpretation of spatial or temporal variation in pollen samples must be done with caution, and we tried to reduce the bias caused by the pollen trapping by avoiding sampling on consecutive days and by operating pollen traps only during four hours each day. Despite the constraints, pollen traps are considered useful for studies of honey bee pollen foraging (GOODWIN & PERRY 1992).

All samples contained pollen from herbs, shrubs, perennials and trees, and it was confirmed that honey bees are generalistic pollen foragers (FÆGRI & PIJL 1980). Even pollen originating from anemophilous plant species, like Poaceae and *Pinus*, were encountered in the samples but none of these pollen types were found in high numbers. Only five pollen types were found in all samples, and a small group of pollen types represented most of the identified pollen in all samples, both when expressed in absolute counts and in relative pollen volume. We decided to transform pollen counts into relative volume estimates, as absolute counts of pollen grains may bias the interpretation of the pollen spectrum (BIESMEIJER *et al.* 1992).

Apart from exposing underlying trends, ordination techniques allow to test whether the observed trends are statistically significant. Secondly, partial ordination may be used to examine the explanatory power of variables after having removed the effect of other variables. The importance of the identified pollen types varied significantly within and between days. *Citrus* and the unknown pollen type were more abundant in the beginning than in the end of the observation period, while *Echium* and *Carduus* became increasingly important at the end of the trapping period. Both *Echium* and *Carduus* are simultaneous nectar and pollen sources, and GIURFA & NÚÑEZ (1992) showed that honey bees visited *Carduus acanthoides* flowers at different development stages during the day and they observed the highest honey bee foraging in those flowers in the early afternoon. Brassicaceae was much more abundant in midday samples compared to morning samples and its abundance did not change during the observation period. *Raphanus raphanistrum* was observed near the bee hive, and honey bees are known to be important pollinators for this species (RUSH *et al.*

1995). However, PERCIVAL (1955) considered this and several other Brassicaceae species as "early morning crops" with maximum pollen presentation around 8 h. Abundance of *Cistus salvifolius* was not found to be associated with either time of day nor early or late part of the observation period. ORTIZ (1994) examined simultaneously the abundance of *Cistus spp.* pollen in honey samples and from the bee hive entrance, and he found *C. salvifolius* pollen to be most abundant in both honey and pollen samples at end of March and beginning of April. *Papaver rhoeas* type was considerably more abundant in morning samples and especially during the late part of the observation period. This is consistent with PERCIVAL (1947), who found that honey bees sampled pollen in this exclusive pollen source from 8 to 11 h.

In addition to the variation within and between days, we found a significant difference in pollen foraging between the two bee colonies. Although there was no significant difference in the amount of pollen collected at the entrance of the two colonies, the qualitative composition of the samples varied significantly. Intra-specific variation in pollen samples from adjacent bee colonies was also shown by SYNGE (1947), SHIMANUKI *et al.* (1967), VISSCHER & SEELEY (1982). WADDINGTON *et al.* (1994) examined the waggle dances in honey bee colonies to outline the spatial distribution of both nectar and pollen sources, and they found significant difference between colonies both in terms of flight distance and in the spatial distribution of visited foraging patches. The two bee colonies exploited a considerable number of minor and major pollen sources simultaneously. This is most likely explained by "probing" of a considerable number of pollen sources in order to constantly assess the relative food quality of potenmtial pollen sources. This may explain the large number of rare pollen types encountered in the present analysis. The present analysis is therefore consistent with the quantitative studies of pollen foraging, which have shown that the pollen foragers respond quickly to the pollen needs in the hive (FREE 1967, HELLMICH & ROTHENBUHLER 1986, BEAUCHAMP 1992, ECKERT *et al.* 1994, CAMAZINE 1993, FEWELL & WINSTON 1996, CAMAZINE *et al.* 1998).

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