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RESEARCH ARTICLE

A COMPARISON BETWEEN PLANT COMMUNITY COMPOSITION AND BOTANICAL SPECIES GATHERED BY HONEY BEES IN A CULTIVATED LANDSCAPE OF WESTERN FRANCE

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ABSTRACT

Botanical inventories were performed in a 1.5 km-radius area around an apiary in a cultivated landscape in North-Western France. Palynological analyses were performed on pollen pellets collected from five colonies of the apiary during one year. Species and phenology of field-inventoried plants were analyzed and compared against the taxa identified in the pollen pellets. Foraging area was characterized by a large cultivated landscape with little -variability in cultivated species and broadly-diverse wild species in grasslands, ditches, roadsides, woods, copses and hedgerows. Palynological analyses found numerous wild-plant pollens throughout the year, with woody perennials predominant at the start of the beekeeping season. The pollens from cultivated species appeared from April and were abundant in spots but little diversified. Taken together, the data indicates that maintaining a high richness and diversity of flowering species over the beekeeping season — which means preserving perennial and natural habitats — may be crucial to provide honeybee colonies with sufficient pollen resources in rural landscapes.

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INTRODUCTION

Recent decades have witnessed unprecedented colony losses in Europe and North America (Aizen and Harder, 2009; vanEngelsdorp and Meixner, 2010). These losses are thought to be driven by a number of causes — (i) infection by pathogens (Guzmán-Novoa *et al.*, 2010; Higes *et al.*, 2010), (ii) poisoning by chemical compounds (Mullin *et al.*, 2010; Henry *et al.*, 2012) and (iii) profound changes in landscape structure and composition induced by agricultural production methods, technological advances and government policies

(Burel and Baudry, 1990; Macdonald and Johnson, 2000; Robinson and Sutherland, 2002). Moreover, recent studies suggest that these biological, chemical and environmental stressors can interact and combine to negatively affect honey bee survival and colony health (Alaux *et al.*, 2010; Vidau *et al.*, 2011; Pettis *et al.*, 2012). Note how modern agriculture is involved in two of these three stress factors. Agricultural intensification has affected land-use patterns and thus shaped landscape structure and composition through major changes, typically increased field sizes (Baessler and Klotz, 2006) with a fragmentation of uncultivated features like forests or hedgerows (Burel and Baudry, 1999), and thus strong decline of natural meadows (Monteiro *et al.*, 2011) with losses of semi-natural elements (Agger and Brandt, 1988). These shifts have been accompanied by increased use of chemical inputs (Robinson and Sutherland, 2002; Baessler and Klotz, 2006), including pesticides, with worldwide production multiplied by a factor of ten from 1955 to 1995 (Carvalho, 2006). In France,

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volumes of pesticides sold have stabilized since 2000 and are currently on the decrease, but the market continues to be flooded with new molecules that are more effective and used at lower doses (Bonney, 2012). These molecules, like all herbicides, are a strong driver of loss of plant diversity (De Snoo and Van der Poll, 1999). Finally, like the widespread use of chemicals, widespread changes in land cover and land-use patterns contribute to an overall homogenization of landscape and vegetation. Many authors have shown how decreasing landscape heterogeneity can dramatically affect on biodiversity through rarefaction of suitable habitats and food resources for many wild species (Robinson and Sutherland, 2002; Benton *et al.*, 2003; Carvell *et al.*, 2007). Wild bees, particularly honey bees and their colonies, are especially vulnerable to such landscape structure and composition changes (Holzschuh *et al.*, 2007; Le Féon *et al.*, 2010; Potts *et al.*, 2010).

Flower richness and abundance and pollen quality are crucial to honey bee food balance and colony health through inputs of pollen molecules that the insect cannot directly synthesize (Louveaux, 1958; Hügel, 1962; Decourtye *et al.*, 2010; Di Pasquale *et al.*, 2013). Low resource quality and quantity, especially pollen resources, is cited as a cause of decreased brood sizes and impaired colony development (Crailsheim *et al.*, 1992; Alaux *et al.*, 2010; De Grandi-Hoffman *et al.*, 2010). To our knowledge, few studies have examined pollen resources exploited by honey bees in rural landscapes (Severson and Parry, 1981; Frankl *et al.*, 2005; Oldroyd, 2007). The objectives of the present study were (i) to define the floristic potential of a foraging area characterized by agricultural practices, (ii) to monitor foraging strategies at both colony scale and apiary scale through pollen pellets analysis, and (iii) to determine the share of cultivated and wild pollens in gathered food resources in order to highlight valuable plants for honey bee colonies in a cultivated landscape context.

MATERIALS AND METHODS

Sampling site and landscape description

This study was performed in 2012 on five honey bee colonies from a sedentary apiary located in Western France (Region of Pays de la Loire, Vendée). The land cover and botanical inventories were performed directly in the field, in a circular 1500 m radius around on the apiary corresponding to an area of 707 ha. The literature suggests that mean foraging distance is roughly 1500 m (Villanueva, 2002; Steffan-Dewenter and Kuhn, 2003) with variability linked to resource type (nectar, pollen, water) season, and location (Visscher *et al.*, 1996; Beekman and Ratnieks, 2000). Plant inventories were performed on landscape entities in the foraging area, on each plot or each linear (hedgerows, boundaries...) where the vegetation was homogeneous. Botanical inventories were performed in representative quadrates of entities, with plants listed and characterized by abundance/dominance index (Braun-Blanquet system; Poore, 1955) and phenological features (floral buds, flowering, fruiting). Sets of inventories were realized over the entire foraging area every two weeks over the whole beekeeping season from February to September.

Biological matrices

Samples of pollen pellets were collected from 5 beehives identified as A, B, C, D and E. The samplings were performed once per month from February to March 2012 and twice per month from April to September 2012. The pollen pellets were harvested on the full floor of the hive located below a wire-mesh floor. This system allowed continuous collection of pollen pellets over a one-month period or a fifteen-day period. Fourteen successive samples of pollen pellets were ultimately collected for each colony. Field-collected samples were immediately placed in ice and stored in a standard freezer at -20°C until analysis. Palynological analyses of pollen pellet samples were performed by Montpellier SupAgro Palynology Unit (France) according to the standard European method (Von der Ohe *et al.*, 2004). Pollens were identified at the various taxonomic levels (family, genus or species) according to pollen grain, and then quantified.

Data processing and statistical analyses

To determine available pollen resources, surface occupied by each inventoried plant was measured via a two-step method. First, the abundance/dominance scores of each plant present in quadrats were converted into cover percentages, considering the central values of the cover range as ordinal scores (Baudière and Serve, 1975). Second, the cover percentages were used to estimate the area of each species in the quadrat, then in homogeneous entity, and finally in the foraging area.

To compare the botanical data and the palynological data, we transformed the floral species identified in the foraging area according to the taxa identified in pollen pellets. Precision of pollen determination and taxonomic level is known to vary with type of pollen. To compare quantities of resources available (flowering plants) and resources exploited (palynological data), some species were regrouped in a same genus or family. Species richness and diversity index were evaluated for species and taxa from botanical and palynological data. Diversity indexes were calculated using Shannon's formula

$$H_s = - \sum p_i \ln(p_i)$$

where “i” refers to a species (or a taxa) and $p_i = \frac{n_i}{N}$ with n_i the number of individual species (taxa) and N the overall number of individuals for all species (taxa). A Spearman's test was used to analyze the correlation between richness of botanical and palynological data. We then performed a multivariate method called Multiple Factorial Analysis. MFA is designed for the analysis of datasets in which individuals are described by several sets of variables. MFA was carried out as follows: an individual was a colony (described by a capital letter) for a fixed period (number 1 corresponds to February, 2 to March, 3 to 1st half of April, 4 to 2nd half of April... and so on until 14 for the 2nd half of September), pollen taxa formed the first set of variables and botanical taxa formed the second set of variables.

This made it possible to analyze the relationships between (i) quantitative plant profiles in the pollen pellets as exploited

resources and (ii) quantitative flowering plant profiles as available resources in the selected foraging area. Botanical taxa and pollen taxa were included as active variables whereas of sampling period was included as a supplementary variable. Managed as a factor analysis, MFA results were co-ordinates, with contribution of initial variables and individuals given for each axis. The common inertia of the two sets of variables was given by the Lg coefficient. The conjunction between botanical taxa and palynological taxa was described by the RV coefficient, with values ranging from 0 (lack of conjunction) to 1 (strong conjunction). Hierarchical Clustering on Principal Components (HCPC) was used to cluster the individuals according to pollen and botanical traits. The statistical analyses were performed with R software (R Development Core Team, 2010) using the FactoMineR 1.16 package (Husson *et al.*, 2007).

RESULTS

Foraging area description

The apiary was located in a rural landscape with a large cultivated area (49% of foraging area) (Table 1) mainly characterized by grain crops and forage crops (respectively 83% and 9%). Protein-rich crops (5%), oil-rich crops (1%) and permanent crops (less than 1%) were a minority. Grassland cover was 22% of foraging area. Woods and copses or urban area occupied less than 10% of foraging area. Length of the hedgerow network was 31 m/ha and length of ditches and roadsides was 32 m/ha.

Table 1. Composition and structure variables of the studied apiary foraging area, giving the main species for each variable

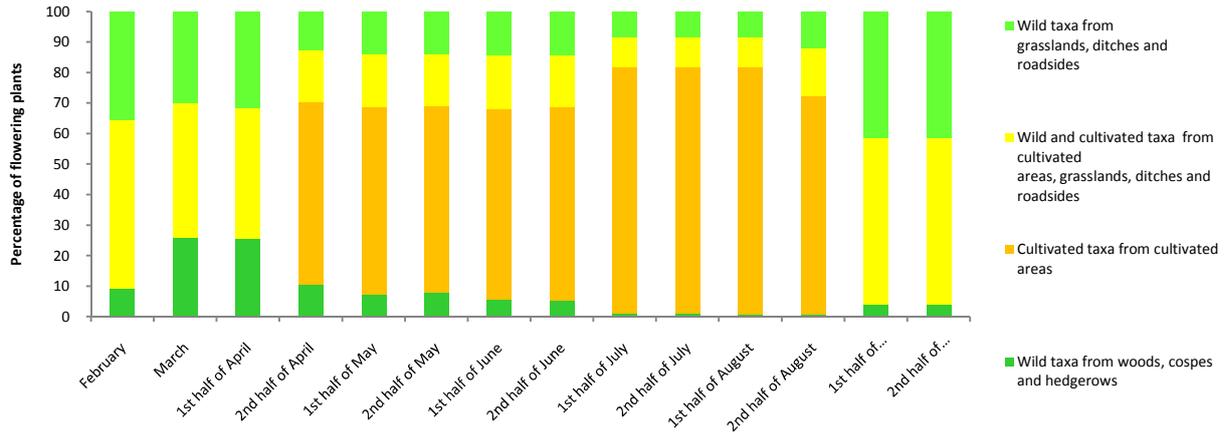
Variables	Apiary foraging	Species
Cultivated area (ha)	346.48	
Grain crops	289.09	<i>Triticum sp.</i> , <i>Zea mays</i> , <i>Secale cereale</i>
Oleaginous crops	3.41	<i>Helianthus annuus</i>
Proteaginous crops	18.72	<i>Pisum sativum</i> , <i>Phaseolus sp.</i>
Forage crops	31.65	<i>Medicago sativa</i> , <i>Lolium perenne</i>
Permanent crops	1.27	<i>Vitis vinifera</i> , <i>Malus domestica</i>
Others	2.34	
Grassland (ha)	156.05	<i>Anthoxanthum odoratum</i> , <i>Taraxacum officinale</i> , <i>Trifolium repens</i>
Wood and copse (ha)	42.46	<i>Quercus sp.</i> , <i>Populus sp.</i> , <i>Alnus glutinosa</i>
Water area (ha)	25.02	
Urban area (ha)	56.11	<i>Prunus sp.</i> , <i>Rosa sp.</i>
Other (ha)	80.88	
Hedgerows (m/ha)	31	<i>Quercus sp.</i> , <i>Rubus sp.</i> , <i>Prunus spinosa</i>
Ditches and roadsides (m/ha)	32	<i>Urtica dioica</i> , <i>Trifolium repens</i> , <i>Taraxacum officinale</i>

The main species in woods, copses and hedgerows were perennial species present year-to-year. A share of the species present in the grasslands was also found in ditches and roadsides. The species present in urban areas were mainly ornamental species. Looking at percentage of flowering surface (Fig 1), the flowering taxa from woods, copses and hedgerows had stronger presence at the beginning of the beekeeping season. Species from grasslands, ditches and

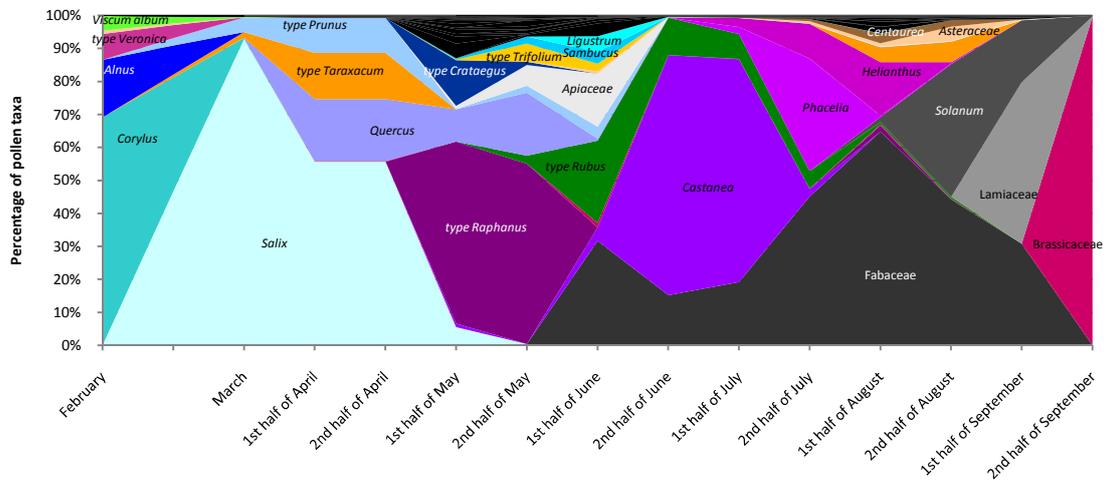
roadsides had stronger presence at the beginning and at the end of the beekeeping season. Cultivated species were dominant from late April to late August. Fifty-four families and 167 species of plants were identified in the foraging area, with 8 ornamental species, 13 cultivated species and 146 wild species (87% of all species identified in the foraging area). At the beginning of the season, from February to March, prominent flowering taxa were wild perennial species such as *Quercus sp.*, *Populus sp.*, *Alnus glutinosa*, *Fraxinus sp.*, or *Corylus avellana*. The cultivated taxa *Triticum sp.* covers the largest area at mid-season (from April to June). In parallel, many perennial species qualifying as permanent crops (*Vitis vinifera*) or wild or ornamental plants (*Ligustrum vulgare*, *Prunus sp.*, *Crataegus monogyna*, *Castanea sativa*, *Sambucus nigra*, *Robinia pseudo-acacia*) were well represented. Later in the season (from July to September), the flowering plants identified were wild perennial or non-perennial species such as *Trifolium sp.*, *Rubus sp.*, *Hedera helix*, *Epilobium hirsutum* or *Lythrum salicaria* and the cultivated species *Helianthus annuus* and *Zea mays*. Some flowering taxa such as *Poaceae*, *Fabaceae*, *Taraxacum sp.*, *Ranunculaceae*, *Plantago sp.*, *Asteraceae*, *Brassicaceae* or *Lamiaceae*, were present throughout the season.

Palynological data

In total, 89 different pollens were identified in pollen pellets and distributed in 51 families. Each colony from the studied apiary gathered pollens from plant species occurring in the same nearby environment, but the five palynological profiles (resources exploited by honey bees throughout the beekeeping season in terms of quality and quantity) were not strictly identical (Appendix A). As expected, many taxa were common to all five honey bee colonies and included *Corylus*, *Salix*, *Taraxacum*, *Prunus*, *Quercus*, type *Raphanus*, *Castanea* or type *Rubus*. This similarity was strictly qualitative, since quantities of shared pollens differed according to colony. Colony-specific palynological profiles were significantly clearer on the basis of minor pollens, i.e. identified in small quantities (< 15% representativity in the palynological profile; Louveaux, 1978). Moreover, some taxa were found in only one colony, such as *Calystegia*, *Cannabaceae*, *Epilobium*, *Ericaceae* or *Hedera* or in some colonies, such as *Fraxinus*, *Genista*, *Lythrum*, *Pinus*, type *Veronica*. A synthetic palynological profile was established from pollens gathered by the five colonies to provide a general overview of plants exploited by honey bees within the studied 707-ha foraging area. The graphs (Fig 2A and 2B) depict the average of the different palynological resources gathered by the five colonies during the brunt of the beekeeping season (February–late September) in North-West France. The synthetic profile showed that wild species were continuously gathered throughout the beekeeping season (from February to September) whereas gathering of cultivated pollen species were intermittently gathered from April to the end of the beekeeping season (Fig 2A). The origin of some taxa such as *Fabaceae*, type *Raphanus* or *Brassicaceae* comprising either wild or cultivated species could not be determined by palynological analyses. From the early beekeeping season until July, the honey bees clearly gathered wild species including several perennials such as *Salix*, *Corylus*, *Alnus*, *Quercus*, type



(A)



(B)

Figure 1. Percentage of flowering plants present in the main land-cover components over the beekeeping season

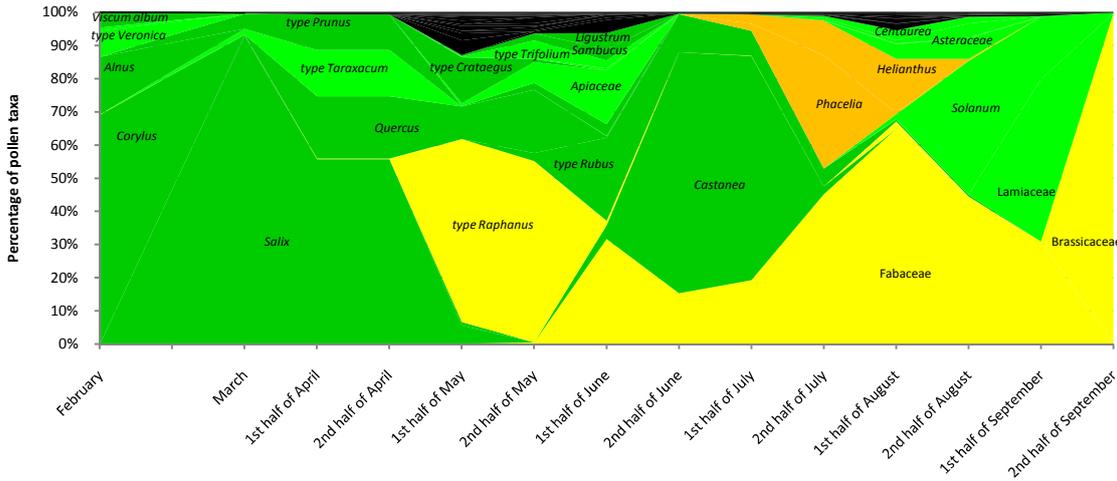


Figure 2. Palynological profile of pellet pollens gathered by five honey bee colonies over the 2012 beekeeping season. A: Annotated pollen taxa annotated and distinguished by distinctive high-contrast color codes. B: Pollens collected from wild perennial plant species colored in dark green, wild herbaceous plant species colored in light green, cultivated species colored in orange, and both wild and cultivated species colored in yellow. Pollen taxa with relative proportions below 5% are colored in black at the top part of the graphs

Crataegus, type *Rubus* or *Castanea* (Fig 2B). From July to September, pellets featured higher proportions of pollens from cultivated plants. During the 2nd half of July, more than 50% of gathered pollen originated from cultivated plants (*Phacelia*, *Helianthus* and potentially *Fabaceae* or *Brassicaceae*). These species represented more than 15% of pollen gathered during the 1st half of August. Wild herbaceous (*Asteraceae*, type *Taraxacum*, *Lamiaceae* or *Centaurea*) taxa were also found, but in much lower quantities. *Zea mays* was identified in palynological profiles from July to September, but quantity of pollen was less than 1% of total pollen sample.

Flowering species versus pollen pellets gathered by honey bees

There was strong discrepancy between the botanical taxa (flowering taxa available in the foraging area) and pollen taxa gathered by honey bees (Fig 3). The number of flowering species was more important than the number of pollens gathered by honey bees.

very beginning and the very end of the beekeeping season. Some species exploited by honey bees were found in the palynological profiles but not inventoried in the field, such as type *Raphanus*.

Table 2. Diversity (Shannon index) of flowering species and pollen taxa during the beekeeping season

	Flowering diversity	Palynological diversity
February	0.33	0.95
March	0.77	0.34
1st half of April	1.02	1.22
2nd half of April	1.07	1.22
1st half of May	1.79	1.66
2nd half of May	1.79	1.63
1st half of June	1.78	1.99
2nd half of June	1.78	0.81
1st half of July	1.52	1.02
2nd half of July	1.52	1.36
1st half of August	1.02	1.36
2nd half of August	1.02	1.29
1st half of September	0.91	1.11
2nd half of September	1.82	0.03

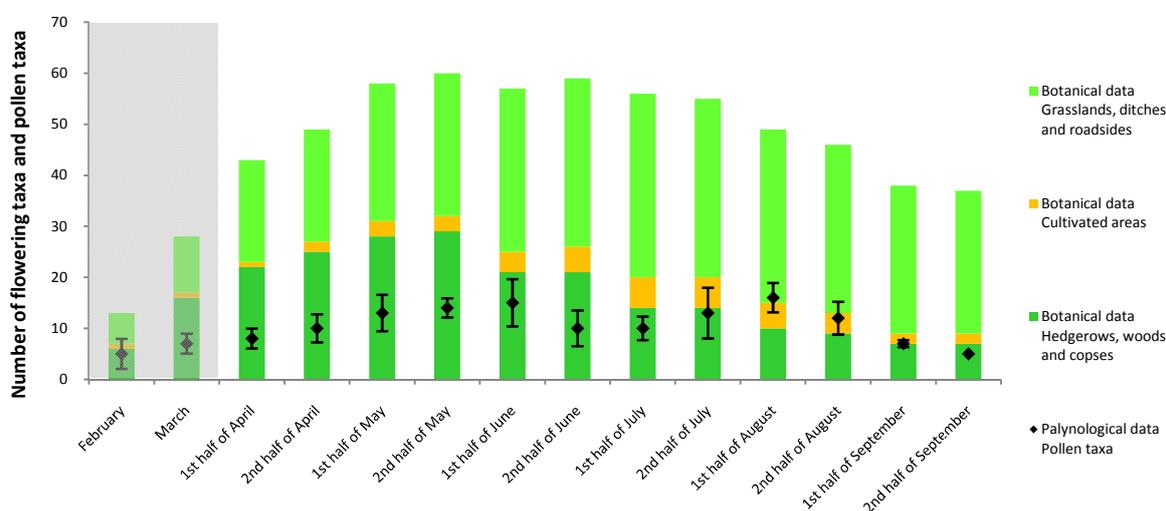


Figure 3. Number of botanical resources (flowering taxa) present in the different categories of land cover and the average number of pollen taxa identified in the colonies during the beekeeping season. The grey area symbolizes the change of timescale

The correlation between the two datasets was significant (Spearman test: $S=16146.31$, $p\text{-value}=4.98.10^{-6}$, $\rho=0.55$). Botanical species richness rose to a peak in May (max=60 flowering species) and June (max=59 flowering species) and then decreased to the end of the beekeeping season. Likewise, the average number of pollen taxa rose to a peak in the 1st half of June (max=22 taxa in a palynological profile) and August (max=20 taxa in a palynological profile) and then decreased. Between these two periods, number of pollen taxa was lower (min=5 taxa in a palynological profile) and was similar to the number of pollen taxa identified during the 2nd half of April. Diversity of flowering species was maximal in May and June, in the spring and during the 2nd half of September (Table 2). Flowering diversity was low at the beginning of the beekeeping season and during August and the 1st half of September. At the same time, diversity of pollen taxa was maximal in May and the 1st half of June but had already tailed off in the 2nd half of June. Pollen diversity was also low at the

At the same time, there were some species identified in-field but not gathered by honey bees, e.g. *Orchidaceae*. The MFA analyzed the link between the flowering species occurring in the foraging area and the families identified in the pollen. Figure 4 shows the first factorial plane of the analysis, which accounted for 17.49% of total inertia. The first MFA axis (Fig 4, Dim 1) was correlated with pollen data (coordinate: 0.73) and botanical data (coordinate: 0.92) and revealed an opposition. Looking at negative coordinate values, there was an association between - flowering perennial species such as *Fraxinus*, *Salix*, *Ulmus*, *Populus*, *Quercus* or even *Alnus*, - pollens gathered from these species (*Salix*, *Quercus* or *Populus*) and - time-periods comprised from February to May (periods 1 to 6). The individuals associated to these pollen and flowering species variables were all five colonies (A, B, C, D, E) for the months of February (period 1), March (period 2) and April (periods 3 and 4). Looking at the positive coordinate values, there was an association between cultivated species

like *Helianthus*, *Z. mays* and wild herbaceous species like *Centaurea*, *Trifolium* or even *Asteraceae* and time-periods from June to September (periods 7 to 14). The individuals associated to these taxa were all five colonies for the months of June (periods 7 and 8), July (periods 9 and 10), August (periods 11 and 12) and September (periods 13 and 14).

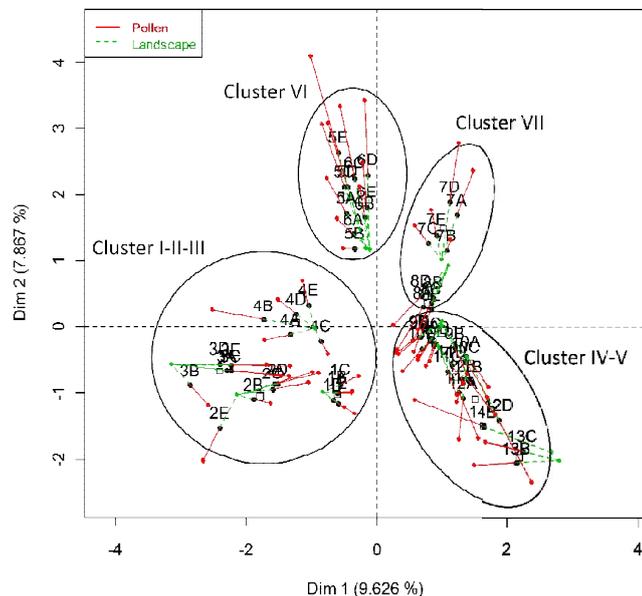


Figure 4. Projections of individuals on the first factorial plane of the Multiple Factorial Analysis. Each point is an individual that represents a colony (described by a letter) for a fixed period (from number 1 to 14 corresponding to February and the 2nd half of September, respectively). Black, green and red points correspond to mean individuals, partial landscape individuals and partial pollen individuals, respectively.

values for common inertia (Lg coefficient) and conjunction between the two types of data (RV coefficients), the correlation between botanical data and palynological data remained highly significant (Lg=1.42, RV=0.42, p-value=4.44.10⁻¹³). Hierarchical Clustering on Principal Components (HCPC) grouped the individuals according to pollen and botanical traits (Table 3). Each cluster included the full set of colonies for a same period. Colonies A, B, C, D and E were characterized mainly by perennial resources such as *Populus*, *Ulmus*, *Corylus* or *Acer* from February to June (period 1 to 6), corresponding to clusters I, II, III and VI drawn on the first factorial plane of the MFA (Fig 4). From July to the end of the beekeeping season (periods 7 to 14), the colonies were characterized mainly by cultivated and wild resources such as *Helianthus*, *Z. mays*, *Hedera*, *Silene*, corresponding to clusters IV, V and VII.

DISCUSSION

Land cover analysis on the foraging area revealed a substantial cultivated area (50% of foraging area), a significant fraction of grassland area (25% of foraging area), and a minor hedgerow network. Landscape context for this apiary can thus be characterized as a rural landscape, particularly a cultivated landscape as defined by Michel *et al.* (2007) for Western France areas.

Available flowering species

While cultivated species occupied a large proportion of foraging area land cover, flowering began in April and peaked over the summer months for *H. annuus* and *Z. mays* and concerned a limited number of taxa. These cultivated surfaces could offer honey bees a significant source of potentially exploitable food at specific developmental stages of the

Table 3. HCPC — the 7 groups of individuals were characterized by pollen traits (“Pollen_” follow-up by the taxa) and botanical traits. Only the most representative taxa are cited in this table. Individual represents a colony for a fixed period

Cluster	Number of individuals	Individuals	Characteristic variables
I	10	2A-2B-2C-2D-2E 3A-3B-3C-3D-3E	<i>Populus</i> / <i>Ulmus</i> / Pollen_ <i>Salix</i> /Araceae /Lauraceae
II	5	4A-4B-4C-4D-4E	Pollen_ <i>Ranunculus</i> / Pollen_ <i>Quercus</i> /Pollen_type <i>Taraxacum</i> / <i>Acer</i> /Pollen_ <i>Liliaceae</i>
III	5	1A-1B-1C-1D-1E	Pollen_ <i>Corylus</i> / <i>Corylus</i> /Pollen_ <i>Alnus</i> /Pollen_ <i>Viscum album</i> /type <i>Taraxacum</i>
IV	21	8A-8B-8C-8D-8E 9A-9B-9C-9D-9E 10A-10B-10C-10D-10E 11A-11B-11C-11D 12A-12D	<i>Helianthus</i> / <i>Zea mays</i> / <i>Silene</i> /Sparganiaceae/Pollen_ <i>Fabaceae</i>
V	3	13B-13C 14B	<i>Hedera</i> /type <i>Cirsium</i> /Alismataceae/ <i>Epilobium</i> /Dryopteridaceae
VI	10	5A-5B-5C-5D-5E 6A-6B-6C-6D-6E	Pollen_type <i>Raphanus</i> / <i>Acer</i> /Pollen_type <i>Crataegus</i> / <i>Robinia</i> / <i>Ilex</i>
VII	5	7A-7B-7C-7D-7E	Pollen_ <i>Apiaceae</i> /Pollen_type <i>Rubus</i> /Pollen_type <i>Papaveraceae</i> / <i>Tilia</i> /Pollen_ <i>Ligustrum</i>

The second MFA axis (Fig 4, Dim 2) was mainly influenced by pollen data. (coordinate values: pollen data 0.87 versus 0.48 for botanical data). This axis showed an opposition between the botanical taxa such as *Robinia*, *Vitis*, Cereals, *Castanea*, *Ligustrum*, *Sambucus* and the pollen taxa such as type *Raphanus*, type *Crataegus*, *Apiaceae*, *Sambucus* on positive coordinate values and botanical taxa like *Viscum album*, *Fabaceae*, *Poaceae*, *Corylus*, *Hedera*, *Z. mays* or even *Brassicaceae* on negative coordinate values. Despite low

colonies (Fewell and Winston, 1992; Dreller and Tarpy, 2000), but they nevertheless represent high-risk areas for honey bees and all pollinators, for several reasons. First, cultivated resources are absent during the first few months of the year that normally coincide with the recovery of beekeeping activity in February and March. During this early-season period, colony development and brood production, which both require protein resources present in pollen (Crailsheim, 1990), cannot rely on cultivated species alone. Second, like most

cereals, cultivated species have unattractively low nutritional value (Louveaux, 1958). At worst, cultivated areas represent a desertic resource. Furthermore, systematic weeding of these crops (botanical inventories), likely involving the use of chemical and mechanical treatments, accentuate this poverty of flowering resources (Freemark and Boutin, 1995). In areas covered by these crops, honey bees have to prolong their flight distance to find sufficient food resources, and these constraints can combine to impact not only the lifespan of foraging honey bees but also the efficiency of resource supply and colony growth (Steffan-Dewenter and Kuhn, 2003). This phenomenon may be amplified by current practices such as crop rotation, where an attractive crop may end up getting replaced in subsequent years by crops that will prove of minor interest for honey bees (Hochörl *et al.*, 2012). Third, these cultivated areas are generally monocultures (mono-crops) that thus provide only a uniform resource for honey bees, and thus only a single protein source, which carries risks of dietary deficiencies and food stress (Brodschneider and Crailsheim, 2010). Likewise, in the current agricultural system, cultivated areas feature a low share of self-propagating plants that cannot compensate for the weight of mono-crops (De Snoo and Van der Poll, 1999). Finally, most cultivated areas are routinely exposed to chemical insecticides, fungicides or herbicides used to control pests and protect primarily cultivated botanical species (Mullin *et al.*, 2010; Lambert *et al.*, 2013). Many studies have demonstrated a chronic sub-lethal toxicity of certain molecules for honey bees and colonies (Belzunces *et al.*, 2012; Henry *et al.*, 2012) and highlighted the risks of foraging cultivated areas. The studied foraging area was nevertheless mainly represented by wild plants, which accounted for over 80% of flowered plant species inventoried. Such diversity may constitute a rich and varied resource for honey bees, especially since recorded flowerings go on over the whole February-to-September beekeeping season, even though diversity of flowering species was variable over the season. These wild plants fall into two main groups: (i) the woody perennial plants found in woods, copses and hedgerows, typically *Quercus sp.*, *Populus sp.*, *Alnus sp.*, or *Prunus sp.*, and (ii) the herbaceous plants present in grasslands, ditches and roadsides, typically *Taraxacum*, *Trifolium*, *Centaurea sp.*, *Asteraceae*. This points to a trade-off between broad time availability on one side but a relatively narrow spatial distribution on the other, since these species are present only in non-croplands, which are poorly represented in cultivated landscapes. Thus potential wild resources in rural landscapes are concentrated in declining fragile areas like grasslands, hedgerows, ditches and roadsides. Indeed, these areas, which might be qualified as “natural” in opposition to cultivated areas, can be maintained while losing all floristic attractiveness due to grazing, grinding or mowing (McLaughlin and Mineau, 1995).

Exploited pollen resources

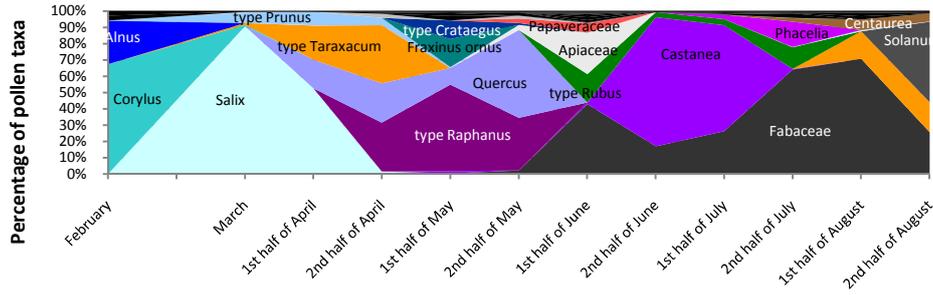
During the beekeeping season and for the studied apiary, honey bees collected a total of 89 different pollens, from 51 families. The average number of counted taxa in this study varies relatively little during the season (from 5 to 16 taxa) compared to the number of taxa present in the foraging area (from 15 to 59 taxa). Variability in the number of taxa registered in pollen profiles is mainly linked to minor taxa

(< 5% of the samples). Beyond number of pollen taxa, type of pollen taxa will change during the beekeeping season according to plant phenology (Sabugosa-Madeira *et al.*, 2008; Aronne *et al.*, 2012; Bagella *et al.*, 2013). In parallel to phenology, the diversity of exploited resources is influenced by several factors, most of which can interact, i.e. floristic attractiveness (Louveaux, 1958), abundance of resources (Suryanarayana *et al.*, 1992) and absence of floristic choice at the beginning or end of the beekeeping season, meteorological conditions, soil quality (Truax *et al.*, 2006), use of chemical soil conditioner or pest control inputs (Billeter *et al.*, 2008), site management (McLaughlin and Mineau, 1995). Assuming that resources exploited by honey bees depend strictly on flowers availability, factors linked to honey bee biology and ecology should also have an influence. First, colony needs evolve with period in the beekeeping season and depend mainly on the intensity of brood rearing (Crailsheim, 1990; Fewell and Winston 1996; Schmickl and Crailsheim 2004; Dreller and Tarpy, 2000). Second, the colony needs a good food balance and good overall health in order for developing and adult honey bees to accomplish physiologically important processes and functions. DeGrandi-Hoffman *et al.* (2010) established a positive correlation between consumed quantity of pollen and development of the hypopharyngeal glands, and Alaux *et al.* (2010) reported that low-diversified feeding was a factor of immunodeficiency for the honey bee. Third, many authors have shown that the honey bee is able to select pollen species according to quality criteria such as the presence of exogenous compounds, i.e. compounds not synthesized by normal honey bee metabolism (Louveaux, 1958; Hügel, 1962). Palynological profiles point to a high ratio of wild plants. Wild-plant pollens were exploited throughout the beekeeping season and represented the exclusive pollen source until May, as shown for pollens of *Corylus sp.*, of *Salix sp.*, of type *Prunus*, of type *Taraxacum*. Furthermore, pollens from woody perennial plants represented a high proportion of the exploited wild species, with specimens (*Corylus*, *Salix*, *Quercus*, *Castanea*) brought to the hive from February to July. As these woody perennial plants are visibly in flower at this period, they appear particularly attractive for honey bees, probably because their pollen presents a nutritional interest for colony development (Louveaux, 1958). Our results point to an essential role for these landscape components — spanning trees/shrubs, like woods, copses and hedgerows — for the domestic honey bee, especially in cultivated areas (Frankl *et al.*, 2005). Other studies have underlined the importance of these “wild” areas not just for communities of auxiliary insects but also for many of their predators (Hinsley and Bellamy, 2000; Millán de la Peña *et al.*, 2003; Aviron *et al.*, 2005).

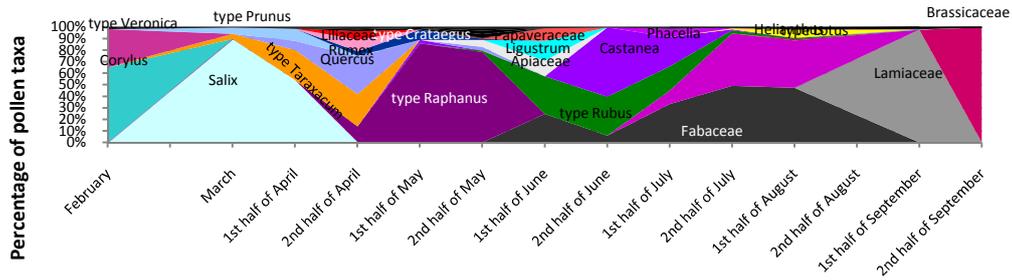
These wild areas are all the more important as they are less affected by chemicals and mechanical treatments (Billeter *et al.*, 2008). Other exploited plant resources include herbaceous species found in grasslands, ditches and roadsides. The pollen profiles reveal that these taxa (*Asteraceae*, type *Taraxacum*, *Lamiaceae* or *Centaurea*) are exploited throughout the beekeeping season, but in lower quantity. The patchy distribution of these taxa within the foraging area presumably makes their discovery and harvest more difficult for honey bees. Pollens of cultivated plants were found almost exclusively in summer months (*Helianthus*). When these

Appendix A: Palynological profiles of pollen pellets gathered by each of the five colonies (A-E) of the test apiary during the beekeeping season. The color code and axis coordinates are the same as those used in Figure 2A.

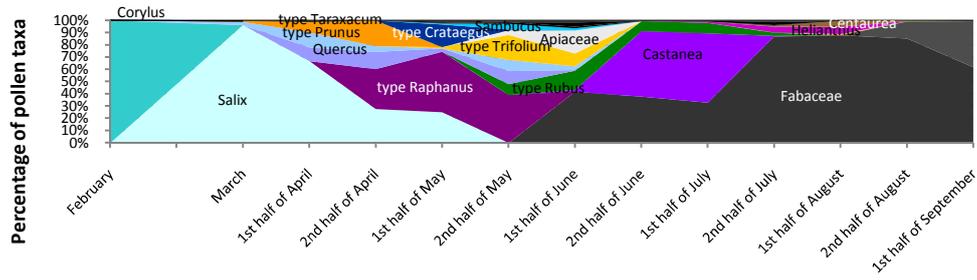
Colony A



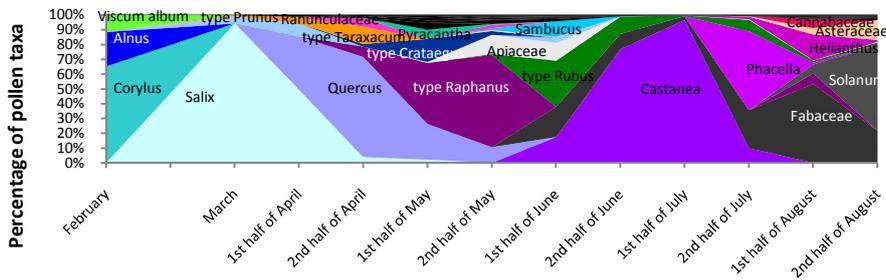
Colony B



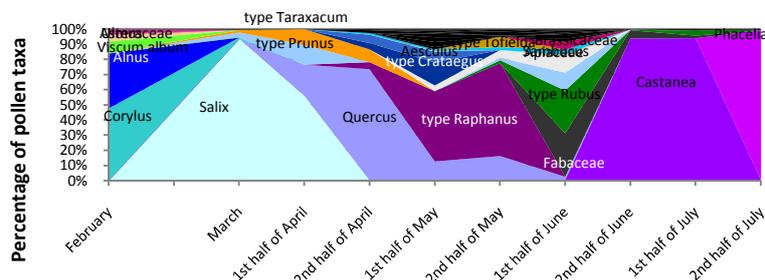
Colony C



Colony D



Colony E



pollens are harvested, it is mainly in high quantity due to the abundance and concentration of the resource. Palynological profiles did however reveal a low quantity of *Zea mays* pollen, suggesting that despite of a large surface cover of maize crops, the honey bees focused on other pollens presumably more valuable for colony health. The apparent disaffection of honey bees for maize, despite its abundance, may well be due to the fact that the foraging area also featured many plant species that more attractive to the bees as they are known to produce pollen compounds of higher nutritive value for the colony (Pohorecka *et al.*, 2013; Höcherl *et al.*, 2012). These plants may have acted as competitors for *Z. mays* in our study. The low proportion of cultivated plants in the pollen profiles throughout the beekeeping season confirms the crucial role of wild species for colony development and long-term survival, especially communities of pollinators, crop auxiliaries and crop-dependent species (Green *et al.*, 1994; Michel *et al.*, 2006).

Due to limitations of the method used for pollen identification, a few taxa could not be characterized, which means relative proportions of pollens of either wild or cultivated origin could not be precisely determined. Based on comparison of the palynological profiles with available phytosociological data, we infer that most of these pollen taxa (especially *Fabaceae* and *Solanum*) corresponded to wild species, supporting the idea that honey bees privileged native floral resources. A similar and even more pronounced behavior has been reported in the case of wild bee communities in semi-natural habitats associated with intensive farming systems (Rollin *et al.*, 2013). Some species known to be highly attractive to honey bees were not found in this study in either botanical inventories of the foraging area or in pollen profiles. Oilseed rape, for example, is a cultivated plant that is widely exploited as a resource when present in the foraging area (Pierre *et al.*, 1999). If present in the studied foraging area, this species would presumably have compensated for the absence of other cultivated species at the end of spring and thus minimized the importance of wild species during the same period. Conversely, had field conditions made it possible to extend the study to mid-October, the pollen profiles would probably have pointed out a dominance of *H. helix*. Indeed, ivy is the main resource exploited by honey bees at the close of summer and before wintering (Jacobs *et al.*, 2010). In conclusion, this study highlights the necessity of maintaining areas of wild perennial plant species such as woods, copses and hedgerows to ensure sufficiently diverse food resources at the beginning of the beekeeping season when honey bees emerge from wintering and when the colony starts working. Wild perennial species offer food resources that are not just quantitatively more diverse but also qualitatively better (as less treated by chemicals and mechanical means) than field crops (which can also change from year to year) and wild herbaceous species. The results also imply that beekeepers should pay particular attention to the choice of the site for setting up a sedentary apiary in landscapes characterized by agricultural practices. Territorial audits might be envisaged as a valuable tool for this purpose.

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