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Original article

Sterol addition during pollen collection by bees: another possible strategy to balance nutrient deficiencies?

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Abstract – Sterols are essential nutrients for bees which are thought to obtain them exclusively from pollen. It is possible that variability in pollen sterol content shapes pollinator-flower relationships, as bee species require the physiological capacity or behavioral adaptations to cope with unfavorable sterolic composition of pollen. One behavioral adaptation used by generalist bees to avoid deficiencies is the mixing of different pollen types from multiple botanical families to achieve an optimal nutritional balance. However, a possible strategy that has never been investigated is the specific addition of nutrients by adult bees to pollen during foraging trips. Here, we analyzed the pollen sterols from 48 plant species and assessed their relation with the level of bee dietary specialization. We also investigated whether sterol addition or modification might occur during pollen collection by comparing hand- and bee-collected pollen for nine bee species. Our results show that sterolic composition tends to be similar within the same plant family, but there was no impact of overall relatedness. For pollen sterol content and bee specialization, pollen from plants used by specialist bees displayed more uncommon sterols than pollen from plants used by generalist bees. In addition, a sterol addition behavior may occur during foraging trips and could be considered a possible strategy to balance nutrient deficiencies. Such behavior was unrelated to bee specialization as this phenomenon was observed in both the generalist *Apis mellifera* and specialist *Dasypoda hirtipes*, suggesting that sterols might constitute a physiological constraint even for specialist species.

pollen / phytosterols / bees / foraging behavior

1. INTRODUCTION

Pollen and nectar constitute the major food sources for most bee species (Danforth et al. 2013). Nectar is the main carbohydrate supply and shows a relatively simple chemical composition (Nicolson and Thornburg 2007), whereas pollen is much more chemically complex and variable, containing in proteins, amino acids, vitamins, and lipids (Roulston and Cane 2000; Villette et al. 2015). Among lipids, sterols are key nutrients extracted from pollen as they are required for numerous physiological processes (e.g., pupation, ovary development, and other reproductive behavior such as caste differentiation in honeybees) and cannot be synthesized de novo by bees (Svoboda et al. 1978; Behmer and Nes 2003). While most of insects use cholesterol ($C_{27}H_{46}O$) as a precursor of the C_{27} ecdysteroid used in the molting process, especially in the predatory clades, sterol requirements are likely

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more complex for bees as pollen contains mostly phytosterols with additional carbons (28- or 29-carbon backbones) (Behmer and Nes 2003). Unlike some herbivorous insects (Behmer and Nes 2003), bees are not able to dealkylate phytosterols (i.e., a reduction of C_{28} or C_{29} sterols into C_{27} intermediate for further production of C_{27} ecdysteroid) and must then synthesize alternative molting hormones with additional carbons, such as makisterone A (C_{28}) (Svoboda et al. 1982). At least 100 different phytosterols have been identified in plants so far (Akihisa et al. 1991) with pollen typically accumulating 24-methylenesterolsand 9, 19-cyclopropyl sterols (i.e., steryl esters) (Lusby et al. 1993), and occasionally sitosterol (Standifer et al. 1968). As far as we know, there is no study exploring the relation between bee host-plant choices and sterol composition of the pollen of host plants.

Specialized species, those foraging on a restricted array of host plants, likely fit tightly with the chemical content of their host plant that might display uncommon nutrients and allelochemicals. In contrast, generalist species have to be adapted to a wider range of chemicals (i.e., nutrients and allelochemicals) that are found across a wider range of plant species (Janz and Nylin 2008). Specialist bees are hence expected to get the steroid precursor for their molting hormone from their preferred hosts, whereas generalist foragers have to cope with a higher diversity and variability of phytosterols, which could therefore be more challenging to meet their physiological needs as well as those of their larvae (e.g., the toxicity of some peculiar sterols, lack of a specific ecdysteroid precursor) (Standifer et al. 1968; Feldlaufer et al. 1993). However, generalists do not forage randomly on all available host plants (Praz et al. 2008; Sedivy et al. 2011) but instead exploit a specific suite of plants to which they might be preadapted behaviorally and/or ecologically (Janz and Nylin 2008; Haider et al. 2013), for instance having all together a suitable sterolic composition (nutrient balancing). Some studies have suggested that pollen mixing behavior in generalist bees could be a strategy to complement nutrient deficiencies and improve diet suitability (e.g., Eckhardt et al. 2013). Such optimization behaviors focused on larval food does may not only comprise pollen mixing but may also include an active component through the specific addition of nutrients from adult bee females. For instance, Svoboda et al. (1986) showed that honeybee workers selectively transfer sterols from their endogenous pools to larval food. This selective sterol transfer (i.e., 24-methylenecholesterol) is performed through the brood material secreted from the hypopharyngeal and mandibular glands and/or the honey stomach. As honeybees usually add salivary enzymes and microorganisms to the pollen stored in their corbiculae (Gilliam 1997), such addition of endogenous sterols might also be carried out during foraging trips, but this behavior has not been investigated to date.

To address these knowledge gaps, we analyzed the pollen from 48 plant species known to be important foraging resources for bees in Northwestern Europe and considered nine bee species (seven specialist and two generalist bees) that forage on at least a subset of the selected plant species. We hypothesized that (i) sterolic composition is conserved among pollen species from a same plant family, (ii) pollen with uncommon sterolic profiles would be more likely to be exploited by specialist bee species (i.e., occurrence of ∂7-sterols that probably requires specific physiological adaptations with peculiar metabolic pathways involved), while generalist bees would forage on pollen displaying more common sterolic profiles (i.e., abundance of the usual 24-methylenecholesterol, ß-sitosterol, and ∂5-avenasterol that fulfill widespread physiological requirements with more generic metabolic pathways involved), and (iii) generalist species transfer endogenous sterols to their pollen loads during foraging trip to complement for potential sterol deficiency, whereas specialized species leave their collected pollen loads unmodified.

2. MATERIALS AND METHODS

2.1. Plant and bee species

Forty-eight plant species from 20 different botanical families were selected. All these species are entomophilous, very common, and constitute important resources of pollen for both generalist and specialist bees in Northwestern Europe

(Michez et al. 2008; Müller and Kuhlmann 2008; Scheper et al. 2014) (Table I).

Apis mellifera and *Bombus terrestris* (Apidae) were selected as generalist bee species that have been reported foraging on the 48 plant species (Kleijn and Raemakers 2008; Rasmont et al. 2008; Leonhardt and Blüthgen 2012; Park and Nieh 2017). Furthermore, seven specialist bees belonging to three different families (Andrenidae, Colletidae, and Melittidae) were selected, each one displaying a restricted host range (see Müller and Kuhlmann 2008 for categories and subcategories): *Andrena vaga* (Andrenidae), narrowly oligolectic on *Salix* genus (Bischoff et al. 2003), *Colletes halophilus* (Colletidae), broadly oligolectic on Asteroideae and Cichorioideae (Müller and Kuhlmann 2008), *C. hederae* (Colletidae), polylectic with strong preference for *Hedera helix* (Schmidt and Westrich 1993), *C. succinctus* (Colletidae), polylectic with strong preference for Ericaceae (Müller and Kuhlmann 2008), *Dasypoda hirtipes* (Melittidae), broadly oligolectic on Asteraceae (Michez et al.2008), *Melitta leporina* (Melittidae), broadly oligolectic on Fabaceae (Westrich 1989) and *M. nigricans* (Melittidae), narrowly oligolectic on Lythraceae (Michez et al. 2008) (Table I).

2.2. Pollen sampling

Floral pollen As field conditions did not allow for easy collection of pollen, we brought back cut plants from sampling locations (Table II) to the laboratory (Belgium, Mons, University of Mons). Pollen was sampled by using a tuning fork to vibrate the stamens and collect pollen from the flowers (around 100 mg of fresh pollen) and cleaned under a binocular microscope (i.e., removal of trichome, anther, dust, or filament). Pollen samples were then lyophilized and stored at – 20 °C prior to chemical analyses.

Bee-collected pollen Pollen loads were sampled in the same plant populations as floral pollen to prevent a potential bias due to natural inter-population variation in sterol composition of pollen. Females from specialist species were caught in order to recover their pollen loads for analysis (around 100 mg) (Table II). These pollen loads are inherently pure or contain minor pollen contaminations (i.e., pollen from non-host plant species) that we considered negligible (Müller and Kuhlmann 2008). For *Apis mellifera*, hives with pollen trap have been placed in the field close to the plant populations or in greenhouses with the specific potted plant species when workers avoid foraging on the target plant species in the field (Table II). As honeybee workers display flower constancy (i.e., foraging only on one plant species during a foraging trip), recovered pollen loads are expected to be unifloral (Leonhardt and Blüthgen 2012). All the pollen loads collected in the pollen trap were then sorted based on their color after checking under a light microscope for pollen origin. As workers of *Bombus terrestris* do not display such constancy (i.e., multifloral pollen loads) (Somme et al. 2015), commercial bumblebee colonies (Biobest *bvba*, Westerlo, Belgium) were placed in greenhouses with potted examples of the target plant species presented successively and separately to ensure pollen load purity (see Table II for details on plant origin). Because of the high variability of worker size, pollen traps are not efficient for bumblebees, and pollen loads were directly recovered by catching the workers during foraging (around 1 g of pollen).

For all pollen samples (i.e., one homogenized pollen sample per plant species and per plant-bee combination), a proportion was removed, and pollen grains were embedded in glycerine gelatin on a slide, and palynological analyses were undertaken to evaluate the monofloral character. The percentages of different pollen types were estimated by counting the grains along three lines randomly chosen across the cover slip at a magnification of × 400 with a light microscope. Pollen types represented by less than 4% of the counted grains were considered contaminants (Müller and Kuhlmann 2008). The pollen grains were identified at magnification of × 400 or × 1000 by comparing their morphology with a set of reference samples (i.e., floral pollen). Following Vanderplanck et al. (2011), each monofloral pollen was then lyophilized and stored at – 20 °C prior to chemical analyses.

Table I. Plant models and associated foragers

Plant family	Plant species	Foragers
Araliaceae	***Hedera helix***	*Apis mellifera* , *Bombus terrestris* , ***Colletes hederae***
Asparagaceae	*Muscari botryoides*	*Apis mellifera* , *Bombus terrestris*
Asteraceae	***Aster tripolium***	*Apis mellifera* , *Bombus terrestris* , ***Colletes halophilus***
Asteraceae	*Cirsium palustre*	*Apis mellifera* , *Bombus terrestris*
Asteraceae	*Heianthus annuus*	*Apis mellifera* , *Bombus terrestris*
Asteraceae	***Hypochaeris radicata***	*Apis mellifera* , *Bombus terrestris* , ***Dasypoda hirtipes***
Asteraceae	***Picris hieracioides***	*Apis mellifera* , *Bombus terrestris* , ***Dasypoda hirtipes***
Asteraceae	***Senecio inaequidens***	*Apis mellifera* , *Bombus terrestris* , ***Dasypoda hirtipes***
Asteraceae	***Senecio jacobea***	*Apis mellifera* , *Bombus terrestris* , ***Dasypoda hirtipes***
Asteraceae	*Solidago gigantea*	*Apis mellifera* , *Bombus terrestris*
Asteraceae	*Tanacetum vulgare*	*Apis mellifera* , *Bombus terrestris*
Balsaminaceae	*Impatiens glandulifera*	*Apis mellifera* , *Bombus terrestris*
Boraginaceae	*Echium vulgare*	*Apis mellifera* , *Bombus terrestris*
Boraginaceae	*Pulmonaria officinalis*	*Apis mellifera* , *Bombus terrestris*
Boraginaceae	*Symphytum officinale*	*Apis mellifera* , *Bombus terrestris*
Brassicaceae	*Brassica napus*	*Apis mellifera* , *Bombus terrestris*
Caprifoliaceae	*Valeriana repens*	*Apis mellifera* , *Bombus terrestris*
Ericaceae	***Calluna vulgaris***	*Apis mellifera* , *Bombus terrestris* , ***Colletes succinctus***
Ericaceae	***Erica carnea***	*Apis mellifera* , *Bombus terrestris* , ***Colletes succinctus***
Ericaceae	***Erica tetralix***	*Apis mellifera* , *Bombus terrestris* , ***Colletes succinctus***
Ericaceae	*Rhododendron ponticum*	*Apis mellifera* , *Bombus terrestris*
Ericaceae	*Vaccinium myrtillus*	*Apis mellifera* , *Bombus terrestris*
Fabaceae	*Cytisus scoparius*	*Apis mellifera* , *Bombus terrestris*
Fabaceae	***Medicago sativa***	*Apis mellifera* , *Bombus terrestris* , ***Melitta leporina***
Fabaceae	*Trifolium pratense*	*Apis mellifera* , *Bombus terrestris*
Hypericaceae	*Hypericum perforatum*	*Apis mellifera* , *Bombus terrestris*
Lamiaceae	*Lamium album*	*Apis mellifera* , *Bombus terrestris*
Lythraceae	***Lythrum salicaria***	*Apis mellifera* , *Bombus terrestris* , ***Melitta nigricans***
Malvaceae	*Tilia cordata*	*Apis mellifera* , *Bombus terrestris*
Orobranchaceae	***Odontites luteus***	*Apis mellifera* , *Bombus terrestris* , ***Colletes hederae***
Papaveraceae	*Chelidonium majus*	*Apis mellifera* , *Bombus terrestris*
Papaveraceae	*Papaver rhoeas*	*Apis mellifera* , *Bombus terrestris*
Resedaceae	*Reseda lutea*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Comarum palustre*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Crataegus monogyna*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Filipendula ulmaria*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Malus pumila*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Prunus avium*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Prunus cerasus*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Pyrus communis*	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Rubus* sp.	*Apis mellifera* , *Bombus terrestris*
Rosaceae s.l.	*Sorbus aucuparia*	*Apis mellifera* , *Bombus terrestris*

Table I (continued)

Plant family	Plant species	Foragers
Salicaceae	***Salix alba***	*Apis mellifera* , *Bombus terrestris* , ***Andrena vaga***
Salicaceae	***Salix caprea***	*Apis mellifera* , *Bombus terrestris* , ***Andrena vaga***
Salicaceae	***Salix fragilis***	*Apis mellifera* , *Bombus terrestris* , ***Andrena vaga***
Salicaceae	***Salix x multinervis***	*Apis mellifera* , *Bombus terrestris* , ***Andrena vaga***
Sapindaceae	*Acer pseudoplatanus*	*Apis mellifera* , *Bombus terrestris*
Sapindaceae	*Aesculus* sp.	*Apis mellifera* , *Bombus terrestris*

Plants visited by one of the targeted bee specialist species are indicated in bold. Plant classification follows Angiosperm Phylogeny Group III (APG III 2009)

2.3. Sterol analyses

Sterol content from both the pollen coat and pollen protoplasm was analyzed using 20-mg samples for floral pollen ($n = 2$–6 analytical replicates per plant species) and bee-collected pollen ($n = 2$–5 analytical replicates per plant-bee combination) according to the method described by Vanderplanck et al. (2011). This method allows for efficient pollen grain disruption, ensuring reliable extraction of sterol from within the protoplasm. Briefly, the main steps of the procedure are (i) saponification with 2 M methanolic potassium hydroxide, (ii) extraction of the unsaponifiable fraction with diethylether and several water-washing, (iii) solvent evaporation, (iv) fractionation of the unsaponifiable by thin-layer chromatography, (v) trimethylsilylation of the sterols (recovered from the silica gel), and (vi) separation by gas liquid chromatography. The total sterol contents for each of the 222 samples were determined considering all peaks of sterols (upper the limit of quantification, LOQ = 9.6 ng/1.2 μl injected) whose retention time was between cholesterol and betulin (internal standard). Individual sterols were quantified on the basis of peak areas from analyses. Identifications were achieved by comparing the relative retention times (ß-sitosterol trimethylsilyl ether = 1.00) with those of oil reference (sunflower oil with well-known composition). These identifications were checked by GC/MS (gas chromatograph/mass spectrometer) analyses (Vanderplanck et al. 2011).

2.4. Statistical analyses

Total sterol contents of floral pollen (i.e., μg/g) from the 48 plant species were compared using a one-way ANOVA followed by a Tukey's multiple comparison test on rank-transformed data ("rntransform" command, R-package "GenABEL"). Differences in sterolic profiles (i.e., relative abundances expressed as g/100 g sterols) as well as in profile of C_{27}-, C_{28}-, and C_{29}-sterols were assessed using perMANOVA (Bray-Curtis dissimilarity index, 999 permutations, "adonis" command) and multiple pairwise comparisons with Bonferroni's adjustment after testing for multivariate homogeneity ("betadisper" command) (R-package vegan, Oksanen et al. 2018). Differences were visually assessed on UPGMA (unweighted pair group method with arithmetic mean) clusters using Bray-Curtis dissimilarity index, and indicator compound analyses were performed to identify sterolic compounds that were indicative of host plants ("indval" command) (R-package labdsv, Roberts 2016). Same analyses were conducted to assess the differences between host plants exploited by generalist and specialist bee species (i.e., two-level factor) (perMANOVA, UPGMA clusters, and IndVal).

To assess the sterol addition behavior during foraging trip (i.e., test if sterolic profile of bee-collected pollen deviates significantly from intraspecific variation), we used pairwise perMANOVA analyses between bee-collected pollen ($n = 2$–5 per bee species) and floral pollen ($n = 2$–5 per plant species) for each plant species. When perMANOVA analyses were significant,

Table II. Sampling locations and dates

Sample	Date	Pollen origin	
Aster tripolium			
Floral pollen	Aug.–Sept. 2011	Netherlands, Zeeland, Hulst	51° 21′ 37″ N, 4° 13′ 4″ E
Apis pollen loads*	Aug.–Sept. 2011	Netherlands, Zeeland, Hulst	51° 21′37″ N, 4° 13′ 4″ E
Bombus pollen loads*	Aug.–Sept. 2011	Netherlands, Zeeland, Hulst	51° 21′ 37″ N, 4° 13′ 4″ E
Specialist pollen loads	Aug.–Sept. 2011	Netherlands, Zeeland, Hulst	51° 21′ 37″ N, 4° 13′ 4″ E
Calluna vulgaris			
Floral pollen	Aug. 2012	Belgium, Anvers, Kalmthout	51° 22′ 48″ N, 4° 13′ 4″ E
Apis pollen loads	Aug. 2011	Belgium, Anvers, Kalmthout	51° 22′ 48″ N, 4° 13′ 4″ E
Bombus pollen loads*	Aug. 2011	Belgium, Anvers, Kalmthout	51° 22′ 48″ N, 4° 13′ 4″ E
Specialist pollen loads	Aug. 2011–2012	Belgium, Anvers, Kalmthout	51° 22′ 48″ N, 4° 13′ 4″ E
Hedera helix			
Floral pollen	Sept.–Oct. 2011	Belgium, Hainaut, Jemappes	50° 27′ 1″ N, 3° 53′ 43″ E
Apis pollen loads*	Sept.–Oct. 2011	Belgium, Hainaut, Jemappes	50° 27′ 1″ N, 3° 53′ 43″ E
Bombus pollen loads*	Sept.–Oct. 2011	Belgium, Hainaut, Jemappes	50° 27′ 1″ N, 3° 53′ 43″ E
Specialist pollen loads	Sept.–Oct. 2012	Belgium, Hainaut, Jemappes	50° 27′ 1″ N, 3° 53′ 43″ E
Hypochaeris radicata			
Floral pollen	Aug. 2012	Belgium, Hainaut, Erbisoeul	50° 30′ 24″ N, 3° 53′ 56″ E
Apis pollen loads	Aug. 2012	Belgium, Hainaut, Mons	50° 27′ 55″ N, 3° 57′ 25″ E
Bombus pollen loads*	Aug. 2012	Belgium, Hainaut, Mons	50° 27′ 55″ N, 3° 57′ 25″ E
Specialist pollen loads	Aug. 2012	Belgium, Hainaut, Erbisoeul	50° 30′ 24″ N, 3° 53′ 56″ E
Lythrum salicaria			
Floral pollen	Jul.–Aug. 2012	Belgium, Hainaut, Baudour	50° 27′ 44″ N, 3° 51′ 29″ E
Apis pollen loads	Jul.–Aug. 2012	Belgium, Hainaut, Mons	50° 27′ 55″ N, 3° 57′ 25″ E
Bombus pollen loads*	Jul.–Aug. 2012	Belgium, Hainaut, Baudour	50° 27′ 44″ N, 3° 51′ 29″ E
Specialist pollen loads	Jul.–Aug. 2011	Belgium, Hainaut, Hollain	50° 32′ 26″ N, 3° 25′ 29″ E
Medicago sativa			
Floral pollen	Jul.–Aug. 2011	Belgium, Hainaut, Nimy	50° 28′ 41″ N, 3° 56′ 50″ E
Apis pollen loads	Jul.–Aug. 2011	Belgium, Hainaut, Lens	50° 33′ 44″ N, 3° 52′ 11″ E
Bombus pollen loads*	Jul.–Aug. 2011	Belgium, Hainaut, Nimy	50° 28′ 41″ N, 3° 56′ 50″ E
Specialist pollen loads	Jul.–Aug. 2011	Belgium, Hainaut, Nimy	50° 28′ 41″ N, 3° 56′ 50″ E
Salix caprea			
Floral pollen	Mar.–Apr. 2011	Belgium, Hainaut, Blaton	50° 29′ 33″ N, 3° 40′ 17″ E
Apis pollen loads*	Mar.–Apr. 2011	Belgium, Hainaut, Blaton	50° 29′ 33″ N, 3° 40′ 17″ E
Bombus pollen loads*	Mar.–Apr. 2011	Belgium, Hainaut, Blaton	50° 29′ 33″ N, 3° 40′ 17″ E
Specialist pollen loads	Mar.–Apr. 2011	Belgium, Hainaut, Blaton	50° 29′ 33″ N, 3° 40′ 17″ E

* Target plant and bee species were placed in greenhouses to ensure pollen loads purity

similarity percentage analyses (SIMPER analyses) were then performed in R using the "simper" function from the vegan package to identify the compounds that were responsible for detected differences between bee-collected pollen and floral pollen. Both similarities and dissimilarities were visually assessed on a non-metric multidimensional scaling (nMDS) ordination using Bray-Curtis dissimilarity index. All analyses were conducted in R (version 3.5.0) (R Core Team 2018).

3. RESULTS

3.1. Variability in pollen sterolic composition among plants

Total sterol content of pollen differed among the 48 plant species (ANOVA on rank-transformed data $F_{47,\ 104} = 13.46$, $p < 0.001$). However, multiple pairwise comparisons did not arrange the different pollen species into clear groups, but pollen from the different species showed a continuum in their sterol content, ranging from 0.9 mg/g (*Muscari botryoides*) to 100.1 mg/g (*Prunus cerasus*) (Fig. 1).

The analyses of sterol composition showed that, most of the time, samples grouped together according to plant species and tended to cluster together at family level, even more at subclass level (Fig. 2a). However, the pollen from some plants displayed a sterolic composition more similar to species belonging to another plant family than to other species within the same family (multiple pairwise comparisons, Table S1, Fig. 2a). Likewise, *Picris hieracioides* was found to cluster apart from the other plants of the *Asteraceae* because of its high and surprising relative proportion in cholesterol (77.1%) (Fig. 2a). Although some sterols are indicative of some plant species (Table III), the results suggest that sterolic composition is not indicative of plant taxonomy per se.

With regard to the carbon backbones, the majority of plants provided pollen with a higher concentration in C_{29}-sterols (i.e., 35 species including *Hypericum perforatum*), but some pollen species displayed very different sterolic profile with a preponderance of C_{27}- (i.e., *Picris hieracioides*) or C_{28}-sterols (i.e., 12 species including *Crataegus monogyna*) (Table III). A few species presented less than 1% of C_{27}- (e.g., *Prunus cerasus*) or C_{28}-sterols (e.g., *Helianthus annuus*), but all displayed at least 4% of C_{29} sterols.

3.2. Pollen sterolic composition and bee specialization

With the exception of *Picris hieracioides*, pollen samples from plants exploited by specialist bees

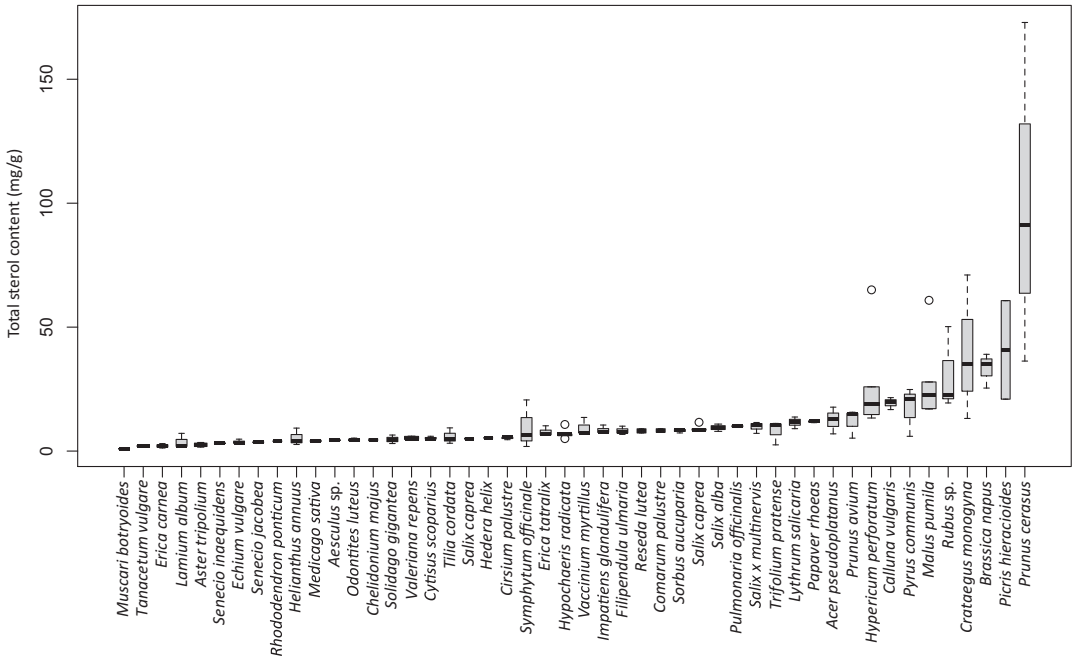

Figure 1. Total sterol content (mg/g) in floral pollen from the 48 plant species. The boxplots show medians (solid line, 50th percentile) and inter-quartile ranges (gray box, 25th and 75th percentiles). The whiskers give the range except for "outliers" (circles) that are more than ± 1.5 times the inter-quartile range larger or smaller than the median.

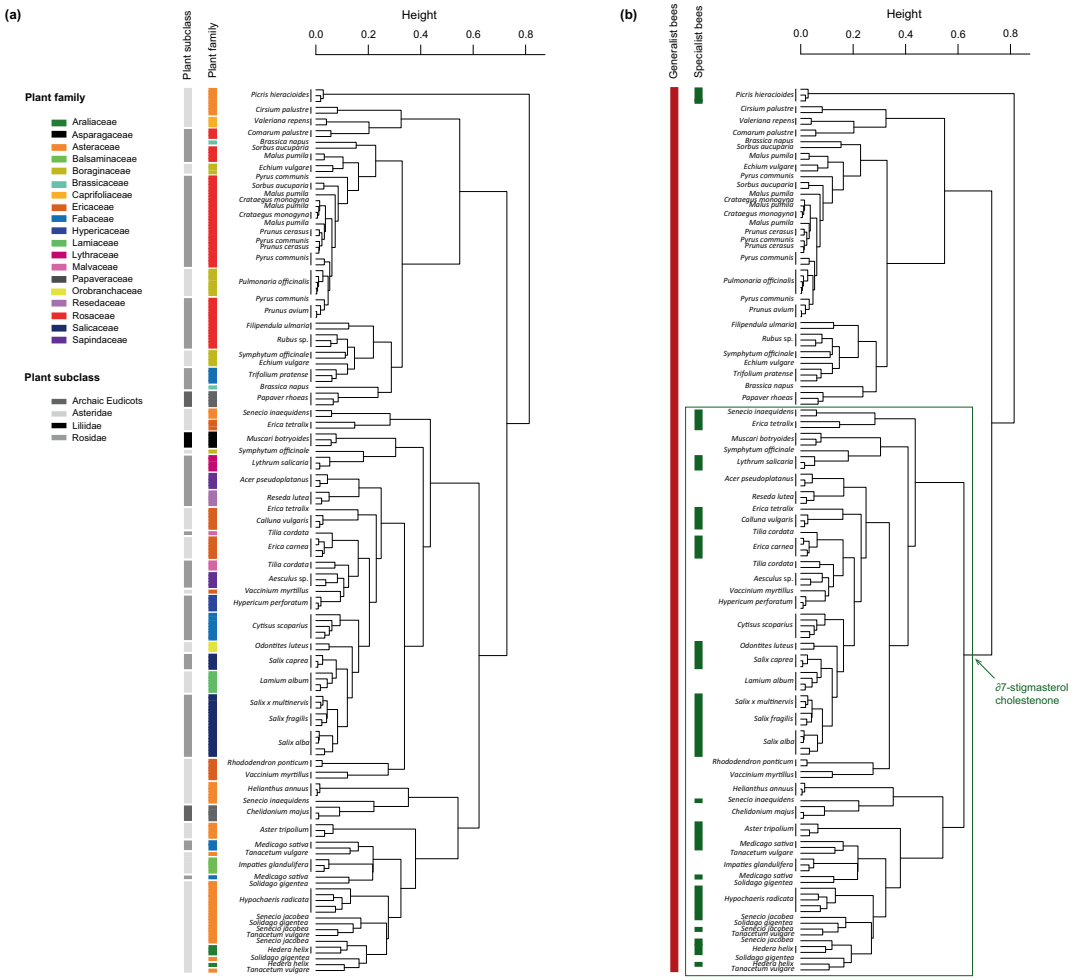

Figure 2. UPGMA cluster using Bray-Curtis dissimilarity index based on sterolic profiles (i.e., relative abundance) from floral pollen. Each branch represents a pollen sample. (**a**) Plant samples from the same taxonomic group (i.e., family or subclass) are marked with the same color. Plant classification follows Angiosperm Phylogeny Group III (APG III 2009). (**b**) Plant species exploited by specialist bees are marked in green, and those exploited by generalist bees are marked in red. The green arrow indicates the indicative sterols from floral pollen foraged by specialist bee species.

clustered on the cladogram (Fig. 2b, Table I). Analyses showed that they were significantly different from plants exploited by generalist bees with regard to proportions of both individual sterol ($F_{1, 203} = 11.07$, $p < 0.001$) and carbon backbones ($F_{1, 203} = 18.10$, $p < 0.001$). While pollen exploited by generalists showed higher proportion of the C_{28} 24-methylenecholesterol (IC = 0.7922, $p = 0.009$), pollen exploited by specialist bees displayed higher proportions of C_{29}-sterols (IC = 0.5621, $p = 0.003$), mainly ∂7-stigmasterol (IC = 0.5751, $p = 0.014$), and C_{27}-sterols (IC = 0.6223, $p = 0.025$), mainly cholestenone (IC = 0.5488, $p = 0.009$) (Fig. 2b). In contrast, total sterol contents of pollen did not differ according to the degree of specialization of foragers ($W = 4359$, $p = 0.269$).

3.3. Sterol addition behavior during foraging trip

Pollen collected by the specialist bee species *Andrena vaga*, *Colletes halophilus*, *C. hederae*,

Table III. Sterolic compositions of floral pollen from the 48 plant models

Plant species (*n*)	C_{27}-sterols			C_{28}-sterol	C_{29}-sterols				
	Cholesterol	Cholestenone	Desmosterol	24-Methylchol. and campesterol	Stigmasterol	ß-Sitosterol	∂5-Avenasterol	∂7-Stigmasterol	∂7-Avenasterol
Araliaceae									
Hedera helix (3)	4.6 ± 3.4	3.6 ± 1.6	1.6 ± 1.7	1.6 ± 1.5	4.9 ± 0.9	29.1 ± 2.1	8.6 ± 1.0	35.8 ± 7.5	10.0 ± 1.6
Asparagaceae									
Muscari botryoides (3)	7.6 ± 3.8	0 .0 ± 0.0	2.7 ± 1.5	35.4 ± 18.0	5.1 ± 2.6	32.9 ± 16.5	5.4 ± 3.1	9.0 ± 4.6	2.0 ± 1.0
Asteraceae									
Aster tripolium (3)	2.1 ± 0.6	1.7 ± 1.0	0.7 ± 0.6	20.2 ± 0.8	4.0 ± 2.6	2.2 ± 0.4	0.5 ± 0.2	41.2 ± 2.0	27.3 ± 0.6
Cirsium palustre (2)	6.2–6.3	24.2–30.1[IC]	7.4–9.1	14.2–17.2	2.3–2. 5	13.1–15.1	1.9–4.1	5.0–6.1	17.4–17.8
Helianthus annuus (3)	2.1 ± 0.3	0.5 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.7 ± 0.4	8.1 ± 0.2	0.0 ± 0.0	78.7 ± 0.9[IC]
Hypochaeris radicata (5)	15.3 ± 3.6	2.7 ± 1.4	1.0 ± 0.5	4.2 ± 0.7	3.25 ± 1.0	17.3 ± 3.6	19.7 ± 0.4	33.7 ± 5.2	2.8 ± 1.5
Picris hieracioides (3)	77.5 ± 38.8[IC]	0.5 ± 0.2	1.3 ± 0.8	0.75 ± 0.8	1.1 ± 0.7	3.7 ± 1.9	9.8 ± 4.9	5.4 ± 2.7	0.0 ± 0.0
Senecio inaequidens (3)	2.4 ± 2.5	0.8 ± 0.7	6.7 ± 6.1	2.3 ± 2.1	2.6 ± 2.3	24.0 ± 12.0	13.9 ± 7.0	16.2 ± 8.6	31.1 ± 17.6
Senecio jacobea (3)	12.3 ± 10.1	2.6 ± 1.4	0.0 ± 0.0	11.6 ± 6.5	2.3 ± 2.2	16.6 ± 12.8	7.9 ± 6.0	37.3 ± 19.9	9.4 ± 4.9
Solidago gigantea (3)	3.3 ± 3.3	0.0 ± 0.0	2.7 ± 1.5	6.2 ± 6.3	2. 6 ± 1.5	17.6 ± 9.5	7.5 ± 5.0	48.1 ± 27.5	11.8 ± 7.0
Tanacetum vulgare (3)	6.3 ± 4.3	0.0 ± 0.0	0.0 ± 0.0	9.4 ± 6.5	2.2 ± 2.5	20.6 ± 11.1	4.2 ± 2.3	48.8 ± 24.9	8.5 ± 4.4
Balsaminaceae									
Impatiens glandulifera (3)	0.2 ± 0.04	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 2.0	0.6 ± 0.5	13.5 ± 0.7	3.5 ± 0.8	50.6 ± 1.8	27.4 ± 1.1
Boraginaceae									
Echium vulgare (3)	1.5 ± 1.2	5.9 ± 0.4	0.2 ± 0.2	66.5 ± 10.3	1.9 ± 0.5	13.1 ± 4.5	7.24 ± 3.2	0.0 ± 0.0	3.7 ± 2.7
Pulmonaria officinalis (5)	2.9 ± 1.2	0.04 ± 0.1	0.4 ± 0.1	89.0 ± 36.3	2.1 ± 1.3	2.9 ± 1.2	2.6 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Symphytum officinale (3)	1.4 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	47.1 ± 26.5	3.6 ± 2.1	24.8 ± 14.8	16.3 ± 11.0	4.4 ± 3.5	2.4 ± 1.4
Brassicaceae									
Brassica napus (2)	1.25–1.6	0.0–0.0	0.0–0.0	43.7–63.9	12.2–14.3[IC]	2.9–3.5	15.5–38.0	0.3–0.7	1.0–1.1
Caprifoliaceae									

Table III (continued)

Plant species (*n*)	C_{27}-sterols			C_{28}-sterol	C_{29}-sterols				
	Cholesterol	Cholestenone	Desmosterol	24-Methylchol. and campesterol	Stigmasterol	ß-Sitosterol	∂5-Avenasterol	∂7-Stigmasterol	∂7-Avenasterol
Valeriana repens (2)	5.7–5.8	5.3–5.7	0.7–0.9	34.2–37.3	4.2–5.0	10.6–12.8	2.8–3.2	3.2–3.4	29.3–29.9
Ericaceae									
Calluna vulgaris (3)	6.3 ± 0.2	4.6 ± 0.2	1.0 ± 0.2	1.6 ± 0.1	3.1 ± 0.7	42.5 ± 0.5	26.7 ± 1.1	3.5 ± 0.4	10.7 ± 0.1
Erica carnea (4)	3.3 ± 0.5	0.3 ± 0.3	0.8 ± 0.3	7.8 ± 0.5	1.2 ± 0.3	51.7 ± 1.0	20.1 ± 0.5	8.0 ± 0.5	6.8 ± 0.5
Erica tetralix (3)	7.6 ± 3.8	1.1 ± 1.4	0.3 ± 0.4	3.8 ± 3.7	1.3 ± 1.6	30.2 ± 3.2[IC]	27.6 ± 10.0	0.8 ± 1.4	27.3 ± 8.6
Rhododendron ponticum (2)	1.0–2.2	0.0–0.0	0.0–0.0	2.4–2.8	0.3–0.9	68.5–69.1	2.0–2.6	18.3–18.4	5.1–6.3
Vaccinium myrtillus (3)	2.5 ± 1.4	0.3 ± 0.4	1.6 ± 1.6	2.7 ± 1.4	0.5 ± 0.4	60.3 ± 31.0	17.2 ± 9.1	3.3 ± 4.7	11.7 ± 9.3
Fabaceae									
Cytisus scoparius (5)	2.5 ± 0.3	0.9 ± 1.4	0.1 ± 0.1	17.4 ± 3.3	0.8 ± 0.3	46.9 ± 3.3	30.2 ± 1.3	0.75 ± 1.0	0.6 ± 0.1
Medicago sativa (3)	1.9 ± 1.5	2.4 ± 1.6	0.1 ± 0.1	5.4 ± 5.1	1.5 ± 1.6	11.1 ± 7.1	8.1 ± 5.75	58.7 ± 30.3[IC]	10.7 ± 7.2
Trifolium pratense (3)	2.6 ± 1.1	2.8 ± 0.1	0.2 ± 0.04	58.0 ± 4.0	2.4 ± 0.7	22.1 ± 1.1	2.8 ± 0.4	1.1 ± 0.5	8.0 ± 2.6
Hypericaceae									
Hypericum perforatum (3)	0.2 ± 0.1	0 .0 ± 0.0	0.0 ± 0.0	6.3 ± 0.3	1.8 ± 0.1	65.2 ± 1.1	24.1 ± 0.4	1.1 ± 0.4	1.3 ± 0.1
Lamiaceae									
Lamium album (4)	5.7 ± 0.4	0.8 ± 0.6	1.2 ± 1.4	3.8 ± 0.8	0.06 ± 0.1	45.5 ± 2.7	42.7 ± 2.1	0.0 ± 0.0	0.2 ± 0.3
Lythraceae									
Lythrum salicaria (3)	0.6 ± 0.1	13.3 ± 1.8	0.1 ± 0.03	28.5 ± 0.4	0.9 ± 0.1	26.3 ± 0.4	24.7 ± 2.6	4.9 ± 0.9	0.8 ± 0.3
Malvaceae									
Tilia cordata (3)	5.1 ± 3.6	1.3 ± 0.7	0.3 ± 0.3	6.0 ± 3.0	2.7 ± 1.4	52.7 ± 26.5	25.5 ± 12.8	2.5 ± 3.3	4.0 ± 2.9
Orobranchaceae									
Odontites luteus (2)	1.2–1.4	1.7–2.0	0.0–0.0	5.7–6.2	5.2–6.0	42.8–46.4	30.8–34.1	5.8–6.6	1.6–2.3
Papaveraceae									
Chelidonium majus (3)	1.0 ± 0.2	0 .0 ± 0.0	0.0 ± 0.0	3.5 ± 5.0	1.7 ± 0.4	9.9 ± 1.0	4.8 ± 0.5	29.1 ± 1.7	50.1 ± 1.8
Papaver rhoeas (3)	7.4 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	55. 4 ± 27.8	0.9 ± 0.8	9.1 ± 4.9	26.4 ± 13.5	0.5 ± 0.3	0.2 ± 0.1
Resedaceae									
Reseda lutea (3)	1.25 ± 0.6	6.8 ± 3.7	0.1 ± 0.1	7.6 ± 3.8	4.8 ± 2.4	31.4 ± 15.7	34.6 ± 17.4	10.0 ± 5.0	3.4 ± 1.7

Table III (continued)

Plant species (*n*)	C_{27}-sterols			C_{28}-sterol	C_{29}-sterols				
	Cholesterol	Cholestenone	Desmosterol	24-Methylchol. and campesterol	Stigmasterol	ß-Sitosterol	∂5-Avenasterol	∂7-Stigmasterol	∂7-Avenasterol
Rosaceae s.l.									
Comarum palustre (2)	2.2–2.7	2.5–6.9	10.1–12.0^{IC}	36.6–37.1	1.9–2.1	19.2–19.7	1.6–3.5	2.2–3.2	17.9–18.4
Crataegus monogyna (3)	0.2 ± 0.1	0.03 ± 0.05	0.0 ± 0.0	95.8 ± 0.9^{IC}	0.04 ± 0.03	2.7 ± 0.6	1.0 ± 0.4	0.0 ± 0.0	0.2 ± 0.04
Filipendula ulmaria (2)	13.9–18.2	0.0–0.0	1.7–2.3	50. 8–57.6	1.6–7.6	10.3–16.5	3.4–3.7	3.2–3.5	2.7–3.2
Malus pumila (5)	0.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	86.9 ± 36.1	0.5 ± 0.6	8.6 ± 5.9	2.5 ± 2.4	0.5 ± 0.4	0.4 ± 0.4
Prunus avium (3)	0.4 ± 0.2	0.2 ± 0.1	0.03 ± 0.03	89.9 ± 45.0	1.1 ± 0.6	5.7 ± 2.9	2.5 ± 1.2	0.0 ± 0.0	0.1 ± 0.1
Prunus cerasus (3)	0.2 ± 0.2	0.04 ± 0.06	0.0 ± 0.0	92.5 ± 46.2	0.1 ± 0.1	5.9 ± 3.1	1.0 ± 0.8	0.0 ± 0.0	0.3 ± 0.2
Pyrus communis (6)	2. 9 ± 2.3	0.0 ± 0.0	0.0 ± 0.0	88.3 ± 33.6	0.3 ± 0.4	5.3 ± 2.5	1.8 ± 1.2	1.5 ± 2.9	0.01 ± 0.01
Rubus sp. (3)	2.1 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	63.8 ± 32.1	1.7 ± 0.9	20.6 ± 10.3	9.3 ± 5.3	1.9 ± 1.0	0.7 ± 0.4
Sorbus aucuparia (3)	2.1 ± 1.1	0.0 ± 0.0	0.1 ± 0.1	81.7 ± 41.0	4.8 ± 7.0	5.2 ± 2.7	5.0 ± 4.1	0.7 ± 0.4	0.5 ± 0.4
Salicaceae									
Salix alba (5)	1.5 ± 0.6	1.4 ± 0.6	5.1 ± 3.6	4.3 ± 1.8	0.5 ± 0.4	52.4 ± 21.4	33.5 ± 13.7	0.3 ± 0.2	1.0 ± 0.4
Salix caprea (3)	1.4 ± 0.7	2.0 ± 1.0	1.1 ± 0.6	4.6 ± 2.3	0.9 ± 0.5	45.7 ± 22.8	41.1 ± 20.6	0.7 ± 0.4	2.5 ± 1.3
Salix fragilis (3)	0.9 ± 0.5	4.9 ± 2.8	0.03 ± 0.02	4.1 ± 2.1	0.1 ± 0.2	50.5 ± 25.3	34.8 ± 17.4	1.7 ± 0.9	3.0 ± 1.5
Salix x multinervis (3)	1.6 ± 0.8	3.8 ± 2.0	1.3 ± 0.7	4.7 ± 2.3	0.9 ± 0.5	48.9 ± 24.4	33.8 ± 16.9	1.3 ± 0.8	3.7 ± 1.9
Sapindaceae									
Acer pseudoplatanus (3)	1.0 ± 2.8	3.5 ± 2.1	0.5 ± 0.2	8.5 ± 0.3	1.6 ± 0.2	34.5 ± 1.2	46.6 ± 0.3^{IC}	1.7 ± 0.1	2.0 ± 0.8
Aesculus sp. (3)	5.0 ± 1.5	0.85 ± 0.9	1.9 ± 3.2	8.1 ± 0.8	2.5 ± 0.3	60.3 ± 2.8	19.2 ± 0.7	1.3 ± 0.6	1.0 ± 0.1

The concentrations of individual sterols are expressed as percentage of total sterolic content (mean ± sd or min-max). Plants foraged by at least one of our specialist bee models are indicated in bold. ^{IC}, indicative compound. Plant classification follows Angiosperm Phylogeny Group III (APG III 2009)

displayed sterolic compositions similar to the floral pollen from their respective host plants (i.e., no modification during the foraging trip) (Fig. 3 Table IV). On the contrary, females of *D. hirtipes* (specialist species) potentially modified the sterolic compositions of pollen collected on *H. radicata* during foraging trip (Fig. 3, Table IV). Analyses showed that pollen loads of *D. hirtipes* were more concentrated in cholesterol (i.e., sterol displaying C_{27} backbone) than pollen from *H. radicata* (SIMPER, contribution to overall dissimilarity, 45.35%) (Fig. 3, Table IV).

With regard to the generalist bee species, sterolic modifications of host pollen might occur during the foraging trip (Fig. 3, Table IV). Compared with floral pollen, pollen loads of *A. mellifera* were more concentrated in cholesterol when workers foraged on *H. radicata* (SIMPER, contribution to overall dissimilarity, 44.65%) but displayed similar sterolic profiles to the host pollen for the other considered plant species (Table IV). In contrast, pollen loads of *B. terrestris* displayed similar sterolic profiles to the host pollen for all the considered plants. Sterol addition behavior potentially differed according to the generalist bee species and the foraged host plant (Table IV).

4. DISCUSSION

When comparing several plant species, floral pollen is highly variable in its chemical composition, including its nutritional content such as sterols (e.g., Vanderplanck et al. 2014; Villette et al. 2015). While such variability can shape bee-plant interactions (e.g., Vanderplanck et al. 2017), whether sterol composition of pollen is related to plant taxonomy and/or to the specialization degree of foragers remains poorly studied. Here, we demonstrated that while pollen from some closely related plant species displayed a similar sterolic profile (e.g., Rosaceae species), dissimilarity in

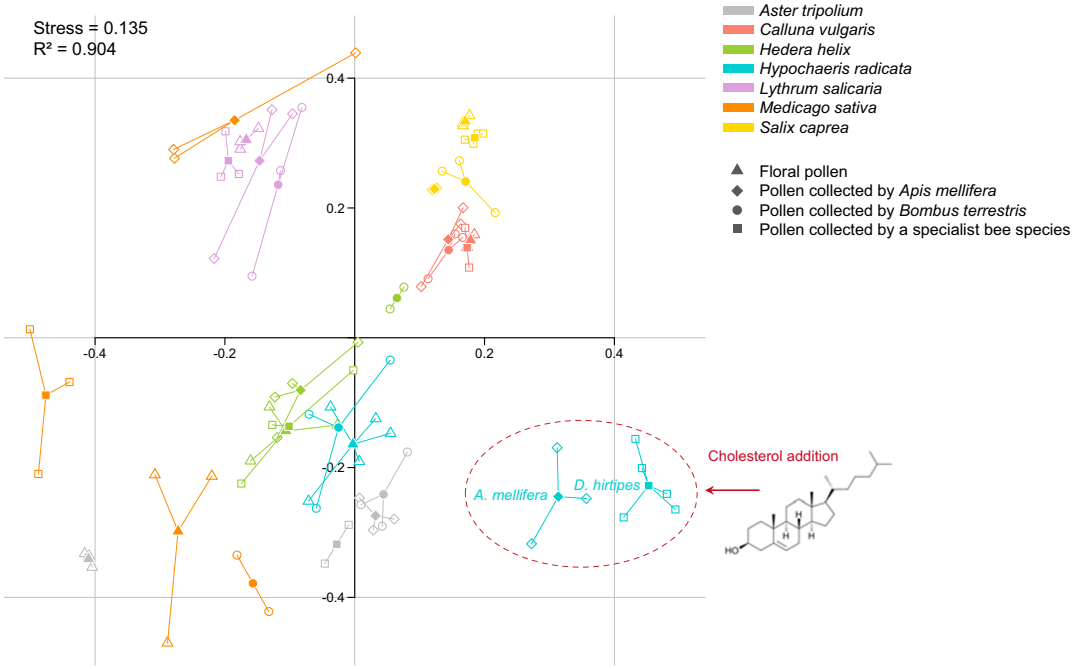

Figure 3. nMDS ordination plot based on Bray-Curtis distances calculated on sterolic profiles (i.e., relative abundance) from floral pollen and bee-collected pollen for each plant species. The red arrow indicates significant modification of sterolic profile between floral pollen and bee-collected pollen. Individual observations are represented by empty symbols and means by full ones.

Table IV. Differences between sterol composition of floral pollen and bee-collected pollen

Plant-bee species combination	Statistics	Additional sterol(s)
Aster tripolium		
Colletes halophilus	$F_{1,3} = 79.657, p = 0.1$	N.a.
Apis mellifera	$F_{1,4} = 140.38, p = 0.1$	N.a.
Bombus terrestris	$F_{1,4} = 82.197, p = 0.1$	N.a.
Calluna vulgaris		
Colletes succinctus	$F_{1,3} = 2.417, p = 0.1$	N.a.
Apis mellifera	$F_{1,4} = 1.739, p = 0.2$	N.a.
Bombus terrestris	$F_{1,4} = 3.111, p = 0.1$	N.a.
Hedera helix		
Colletes hederae	$F_{1,4} = 0.420, p = 0.7$	N.a.
Apis mellifera	$F_{1,5} = 1.477, p = 0.298$	N.a.
Bombus terrestris	$F_{1,3} = 9.210, p = 0.1$	N.a.
Hypochoeris radicata		
Dasypoda hirtipes	$F_{1,3} = 100.05, p = 0.012$*	Cholesterol
Apis mellifera	$F_{1,6} = 33.658, p = 0.018$*	Cholesterol
Bombus terrestris	$F_{1,6} = 0.775, p = 0.51$	N.a.
Lythrum salicaria		
Melitta nigricans	$F_{1,4} = 15.744, p = 0.1$	N.a.
Apis mellifera	$F_{1,4} = 5.098, p = 0.1$	N.a.
Bombus terrestris	$F_{1,4} = 7.187, p = 0.1$	N.a.
Medicago sativa		
Melitta leporina	$F_{1,4} = 6.386, p = 0.1$	N.a.
Apis mellifera	$F_{1,4} = 14.54, p = 0.1$	N.a.
Bombus terrestris	$F_{1,3} = 3.910, p = 0.1$	N.a.
Salix caprea		
Andrena vaga	$F_{1,5} = 5.484, p = 0.055$	N.a.
Apis mellifera	$F_{1,3} = 29.658, p = 0.1$	N.a.
Bombus terrestris	$F_{1,4} = 5.848, p = 0.1$	N.a.

Results from perMANOVA analyses are indicated for the different pairs of bee and plant species (SIMPER analysis when significant). Specialist bee species are indicated in bold

pollen sterolic composition might also occur between closely related plant species (e.g., Boraginaceae with *Pulmonaria officinalis* and *Symphytum officinale*). The fact that taxonomy cannot be fully used to predict pollen sterol profile has been already observed in the family Salicaceae (*Populus* and *Salix*, Standifer et al. 1968). The sterol analyses led on floral pollen from 48 plant species revealed that ß-sitosterol, ∂5-avenasterol, and 24-methylenecholesterol are widespread phytosterols in pollen. These C_{28}- and C_{29}-sterols are already known to be very common in plant sterolic profile (Behmer and Nes 2003). In contrast, C_{27}-sterols and ∂7-sterols are much more uncommon in plants (Behmer and Nes 2003) but were found in the pollen from some targeted species (e.g., Asteraceae).

Growing evidence shows that suites of chemical traits (e.g., pollen nutrients but also other nectar chemicals) could be an integral part of pollination syndromes (Johnson et al. 2006; Weiner et al. 2010). Although the functional

significance of pollen sterol variation is poorly understood, it may drive (at least partly) the floral choices of bees and then their interactions with surrounding plant species. Regarding the evolution of plants, the presence of particular sterols could promote foraging specialization of bees like it does for some phytophagous species (e.g., *Drosophila pachea*, Heed and Kircher 1965) and like secondary metabolites in nectar do for many pollinators (Adler 2000). In addition, there could be a clear evolutionary advantage for plants to attract specialist pollinators as it ensures effective pollination through species-specific pollen deposition in some situations (Schlindwein and Wittmann 1995; Waser et al. 1996). In our study, we observed that specialist species forage on plants that provide pollen with some particular sterols such as cholesterol and desmosterol, which have 27-carbon backbone and are uncommon plant sterols (e.g., Melittidae). Such selection of pollen displaying a particular sterolic composition may reflect the need for a given sterol to meet physiological requirements. Although this hypothesis is congruent with recent study suggesting that pollen sterols may have driven host shift during bee evolution (Vanderplanck et al. 2017), studies led on additional bee species, especially on specialist species foraging on other plant families than those considered herein (e.g., *Andrena pandellei*, a specialist of Campanulaceae, Westrich 1989), are still needed to fully corroborate it. Moreover, further studies on diversity of molting hormone in bees should allow for establishing physiological pathways and better understanding the role of sterols in plant selection by pollinators and bee evolution.

To meet their physiological requirements, generalist bees can display different strategies: (i) pollen mixing behavior (Eckhardt et al. 2013); (ii) preferentially choosing, collecting, or communicating diet quality to regulate nutrient intake (Hendriksma and Shafir 2016; Vaudo et al. 2016b; Zarchin et al. 2017; Hendriksma et al. 2019); (iii) physiological adaptations to digest pollen from a large array of host plants (Sedivy et al. 2011). Here, we considered a new potential behavior: sterol addition behavior during foraging trips. Our data show that pollen loads of generalist bees (i.e., *A. mellifera* and *B. terrestris*) did not always display the same sterolic composition than the floral pollen they foraged on. Although we cannot exclude potential inter-floral, inter-individual, or inter-population variations, though we avoided them as much as possible, our results suggest that generalist bees might modify the sterolic content of pollen during foraging trip, these modifications depending on both bee and plant species involved. Such differences in nutritional content have been already shown between floral and stored pollen in previous studies on *A. mellifera* (Human and Nicolson 2006). However, our data indicate that this modification might occur additionally before storage in beehive, during the foraging trip. Directly after its collection, the gathered pollen undergoes a dynamic process that may have an important function in nutrient processing. Actually pollen packed for transport is inoculated with microbes, nectar, and salivary enzymes from the forager itself (i.e., mandibular and hypopharyngeal gland secretions) (reviewed in Anderson et al. 2011). Such additions could result in both microbiological and biochemical changes in collected pollen, as observed for stored pollen. These changes are primarily due to fermentation of added sugars by bacteria and fungi (Louw and Nicolson 1983; Winston 1993). Moreover, these microorganisms also produce various chemicals such as lipids that may contribute to chemical changes of pollen (Gilliam 1997), in addition to the sterols contained in the glandular secretions of bees (Svoboda et al. 1986; Pianaro et al. 2009; Ferreira-Caliman et al. 2012). For social bee species such as *Apis mellifera*, further modifications might even occur in stored pollen as corbicular pollen pellets are coated with additional layers of nectar, honey, and oral secretions when packed into cells (Roulston and Cane 2000; Nicolson 2011). Such modifications that improve pollen nutritional quality and palatability could appear less important in honey bees due to the lower importance of stored pollen in larval nutrition compared with solitary bee species as honeybee larvae are mainly fed with jelly. However, young adult workers consume stored pollen to complete their adult development, especially to develop secretory glands (i.e., hypopharyngeal glands used to produce food secretions), and nutrients reserves are critical for later roles as nurses

and foragers (Haydak 1970; Crailsheim et al. 1992). Moreover such sterol addition by honeybee foragers could allow nurses to gain essential sterols for larval development. Indeed, honeybee nurses are in charge of larval nutrition but do not forage outside the colony and are then not able to adjust for nutrient deficiencies using abovementioned strategies as they can only get sterols from stored pollen. This highlights the importance of stored pollen that should then be complemented for potential nutrient deficiency.

Regarding plant species-specific sterol modification of pollen by generalist bees (i.e., *Apis* and *Bombus*), such a hypothesis suggests a transfer on purpose, meaning that bees would be able to identify a specific sterol deficiency and complement for it. Recently, research has focused on whether foraging bee can determine the quality of pollen sources offered by plant species (Robertson et al. 1999; Hanley et al. 2008; Leonhardt and Blüthgen 2012; Somme et al. 2015). Since foragers individually forage and assess resource levels inside the nest, one might predict that it would be more efficient for social bees to possess the ability to individually assess the pollen quality directly at the flower level (Nicholls and Hempel de Ibarra 2017). While Pernal and Currie (2002) have stated that the use of oral or alimentary receptors to rapidly assess pollen quality is unlikely, it has been suggested that gustatory sensilla on the mouthparts of honeybee might be responsive to macronutrients (de Brito Sanchez 2011 and references therein). Foraging bees may actually have opportunity to sample grains pre-ingestively during foraging using their main gustatory organs, the mouthparts, and antennae (Nicholls and Hempel de Ibarra 2017). Although many studies have focused on protein detection, some studies have suggested that other macronutrients such as lipids (i.e., including sterols) are either equally or even more important (e.g., Vaudo et al. 2016a). This could lead to bee abilities for detection of sterol deficiency and subsequent selective addition of sterols from endogenous pool. However, such a hypothesis requires further investigation focusing on bee physiology and using specific methodologies for validation.

While specialist bee species are expected to be tightly adapted to the specific chemical composition of their host pollen (Weiner et al. 2010; Sedivy et al. 2011), our results highlighted a difference in the sterolic composition of pollen loads of *Dasypoda hirtipes* compared with the pollen of its host plant. Although we do not have information on intraspecific variation (i.e., intrafloral, intra-individual, and intra-population variations), our data suggest that this melittid species might add C_{27}-sterol to its host pollen while foraging (i.e., cholesterol for *Dasypoda hirtipes,* $15.3 \pm 3.6\%$ in host pollen vs $59.4 \pm 5.8\%$ in pollen loads). This sterol addition may originate from glandular secretions of bees. For instance, cephalic glands are known to play a role as storage organs of phytosterols (Svoboda et al. 1986; Pianaro et al. 2009; Ferreira-Caliman et al. 2012). In Melittidae, the hypertrophied Dufour's gland may be especially involved in such sterol addition after pollen collection. This gland is known to produce secretions extremely rich in diverse natural products (Hefetz 1987), including traces of cholesterol (Mitra 2013). The assumption that the Dufour's gland may be involved in sterol addition Melittidae is supported by the larval nutritional function of Dufour's gland secretions that have been already described in *Anthophora* , *Emphoropsis* , and *Megachile* bees (Norden et al. 1980; Cane and Carlson 1984; Duffield et al. 1984; review in Mitra 2013).

Our study highlights the importance of pollen sterols in the interactions between bees and plants. Several studies have shown that bee females display multiple strategies to mitigate unfavorable pollen properties and feed their larvae with suitable food (Sedivy et al. 2011; Eckhardt et al. 2013). Our results suggest the possibility that some bee species may add sterolic compounds during foraging trips, likely to meet physiological requirement related to molting. Such behavior in specialist foragers challenges the hypothesis that specialist species are more competitive on sub-optimal diets since they are expected to fit tightly with the nutritional and allelochemical content of their host without any modification (Janz and Nylin 2008). Investigating the molting process in bees will help elucidate how pollen sterolic content drives host-plant selection and its implication in bee-plant evolution.

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AUTHORS' CONTRIBUTION

DM and MV conceived the project. DM, GL and MV designed the methodology. PLZ and MV collected and analyzed the data. DM and MV led the writing of the manuscript. PLZ and GL contributed critically to the drafts and gave final approval for publication.

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L'ajout de stérol lors de la récolte du pollen par les abeilles : une autre stratégie possible pour équilibrer les carences en nutriments ?

pollen / phytostérols / abeilles / comportement de recherche de nourriture.

Die Zugabe von Sterol während des Pollensammelns von Bienen: eine mögliche anderweitige Strategie zum Ausgleich von Nährstoffdefiziten?

Pollen / Phytosterole / Bienen / Sammelverhalten.

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