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The same, but different: pollen foraging in honeybee and bumblebee colonies

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Abstract – Like many other bees worldwide, honeybees and bumblebees are threatened by human-induced disturbances. Yet some species decline and others thrive, likely due to different foraging strategies. As little is known about how resource intake translates into nutrients available to colonies, our study aimed to better understand how differences in foraging strategies may affect colony health by relating differences in pollen spectra collected to differences in nutrient composition. The *Apis* and *Bombus* colonies studied were all located at the same site, but nevertheless differed in the spectra of plant species visited for pollen collection and the quality of pollen collected. Bumblebees generally collected pollen with significantly higher pollen protein content and more essential amino acids. Unlike honeybees that tend to exploit large resource patches, bumblebees thus seem to select the "better" pollen and to focus on quality instead of quantity.

generalist pollinators / amino acids / floral resources

1. INTRODUCTION

It is now widely recognized that bees are threatened by human activities (Banaszak 1995; Biesmeijer et al. 2006; Kosior et al. 2007; Winfree et al. 2009; Potts et al. 2010; Williams et al. 2010; Cameron et al. 2011). Among these, changes/reductions in habitat and resource availability and diversity are considered one (albeit not the only) cause of the observed bee decline (Williams 1986; Steffan-Dewenter and Tscharntke 1999; Aizen and Feinsinger 2003; Goulson and Darvill 2004; Hines and Hendrix 2005; Goulson et al. 2008; Winfree et al. 2009). Both larvae and adults are highly dependent on floral resources because they obtain their entire caloric energy from flowers (Michener 2007). Bee diversity and abundance

consequently decrease with decreasing plant diversity (Biesmeijer et al. 2006).

Generalist pollinator species are commonly considered less vulnerable to losses of plant diversity because their generalist foraging behavior is thought to enable them to switch from one plant species to another more easily than specialists (Johnson and Steiner 2000; Aizen and Feinsinger 2003; Biesmeijer et al. 2006; Steffan-Dewenter et al. 2006; Devictor et al. 2008; Klein 2011, but see Bommarco et al. 2010 and Winfree 2010). Among generalist bees, social bees play a dominant role as plant pollinators due to the numerous numbers of foragers reared by a single nest. Social bees, in particular honeybees (*Apis mellifera*) and bumblebees (*Bombus* species), also rank among the top crop pollinators (Klein et al. 2007a, b). Honeybees are threatened by as yet unknown causes (National Research Council 2007; Williams et al. 2010). Several bumblebee species have also strongly declined in recent years and

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even partly become extinct from some areas, whereas others seem to thrive (Williams 1982; Goulson et al. 2005; Williams et al. 2007, 2009; Bommarco et al. 2011; Cameron et al. 2011). The observed discrepancies in the abundance and vulnerability of different bumblebee species have been related to differences in morphology, ecology, and foraging behavior (i.e., tongue length (Goulson et al. 2005), foraging range (Benton 2006), colony size (Rundlöf et al. 2008), climatic niche (Williams et al. 2007; 2009), emergence time of queens (Fitzpatrick et al. 2007; Williams et al. 2009), and the degree of floral specialization (Williams 1989; Goulson and Darvill 2004; Xie et al. 2008)). These factors directly or indirectly affect the intake and exploitation of floral resources. However, most studies addressing the vulnerability of social bees are highly correlative, basically relating bee abundance to floral resource abundance in the field. Consequently, we still do not fully understand how resource availability and diversity as well as their interactions with nutrient quality affect the fitness of bees and why they affect different species differentially. For instance, how resource availability and diversity translate into nutrients available to a colony has as yet been barely addressed. This knowledge is, however, essential for understanding the correlation between available resources, foraging behavior, and colony health. Many (albeit not all) bumblebee species and honeybees are generalist foragers with regard to pollen and nectar. While nectar represents the major energy source of adult bees, pollen is considered the essential component of bee nutrition as it is consumed by both adult bees and larvae (Haydak 1970; Dietz 1992). Pollen not only provides mainly protein, but also lipids, vitamins, and minerals (Herbert 1992). With regard to protein, different plant species vary considerably in total protein content and amino acid composition (Roulston et al. 2000; Weiner et al. 2010). They are, however, qualitatively similar (Roulston and Cane 2000; Weiner et al. 2010) with regard to amino acids considered essential for honeybees (according to de Groot 1953) and most likely also bumblebees (Génissel et al. 2002).

Notably, studies that have investigated floral resources used by bumblebees were mainly focused on foragers observed at flowers in the field (Williams 1989; Goulson and Darvill 2004; Peat et al. 2005; Kleijn and Raemakers 2008; Xie et al. 2008; Connop et al. 2010). Fewer authors have studied resource allocation directly at the bees' nests (e.g., Kratochwil and Kohl 1988; Peat and Goulson 2005; Munidasa and Toquenaga 2010). Assessing resource use of social bees in the field is, however, prone to sample biases as individual bees tend to (at least periodically) specialize on particular plant species (Heinrich 1976; Heinrich et al. 1977; Kratochwil and Kohl 1988) and because colonies are relatively long-lived, which increases the spectrum of plant species used for resource allocation. Some authors tried to overcome this field work-related bias by sampling greater numbers of bees to get a representative estimate of the plant species used, by sampling pollen loads throughout the lifetime of a species' colonies, and by collecting different species from the same areas to make sure that they have the same choice of flowering plant species (Williams 2005; Kleijn and Raemakers 2008). However, monitoring resource intake at the colony level of species found in the same habitat may still provide the most complete picture of—as well as of species-specific differences in—the floral and hence nutritive spectrum used by foraging social bees (Kratochwil and Kohl 1988).

Our study aimed to better understand how differences in resource intake may affect honeybee and bumblebee colonies. We, therefore, investigated (1) whether colonies of honeybees and two different bumblebee species located at the same habitat use the same or different plant spectrum for pollen collection and (2) whether differences in the plant spectra used translate into nutritional differences with regard to total protein content and amino acid composition.

2. MATERIALS AND METHODS

2.1. Study sites, colonies, and sample collection

All colonies were located in proximity to the Biozentrum of the University of Würzburg in an

approximately 10,000-m^2 grassland area with hedges and fruit trees. They were placed close to brushwood for protection against extreme weather conditions (i.e., direct insolation, heavy wind and rain). The area was surrounded by gardens, fields, small forest patches, and further grasslands and hedgerows, hence representing a relatively heterogeneous landscape with ample forage opportunities.

Pollen was collected from two managed honeybee colonies (kindly provided by the Bee Group of the University of Würzburg), from two *Bombus pascuorum* colonies (established by wild queens in bumblebee nest boxes purchased from Schwegler-und-Vogel Naturschutzprodukte, Schorndorf, Germany) and from two *Bombus terrestris* colonies (obtained from re-natur, Ruhwinkel, Germany).

Pollen sampling took place in the morning (between 8 and 11 am) of overall 14 nonrainy days in July and August 2010. We used pollen traps to collect pollen from honeybees. The traps were attached to the hive entrances for 60–90 min, forcing bees to squeeze through little holes (5 mm in diameter), thereby removing and collecting pollen loads from hindlegs of returning foragers. We tried to establish a similar system for bumblebee colonies, but failed to remove pollen loads from their legs with pollen traps due to bumblebees' intracolonial size polymorphism. We, therefore, captured returning foragers at nest entrances using insect nets. Because bumblebee colonies never reached the same workforce as honeybee colonies, we were able to catch all returning foragers within a given time frame (60–180 min). Pollen foragers were immobilized in a fridge (3–7 min). Their pollen loads were removed using forceps. Colors of pollen loads from each returning forager were noted.

For honeybees, we collected the same amount of pollen (as we had obtained from bumblebees) from the pollen traps attached to the colonies' nest entrances by randomly picking an equivalent amount of pollen loads.

Pollen loads from honeybees and bumblebees were then transferred to clean Eppendorf tubes. For each bumblebee forager, the pollen from one hindleg was kept in one Eppendorf tube (for plant source identification and measurement of sugar content), whereas the pollen from the other leg was mixed with pollen from all foragers caught at a particular day to

obtain a broader picture of the overall pollen intake (for amino acid analysis). For honeybees, we treated two pollen balls of equal color and size as pollen load from one forager and sorted them accordingly. Single and mixed pollen loads were weighed (wet weight) and then stored in a freezer.

2.2. Plant source identification by pollen grain analysis

To identify plant sources of pollen loads obtained from honeybees and bumblebees and to analyze the degree of floral specialization of individual foragers, permanent glycerine–gelatin microscope slides were prepared for all single pollen loads collected from returning foragers. One milliliter of concentrated sulfuric acid/acetic acid anhydride (1:10) was added to pollen loads in Eppendorf tubes, mixed, and heated at 98°C for 2 min. The pollen acid mixture was subsequently centrifuged (1 min) and the supernatant discarded. We then added 1 mL of distilled water, mixed water and pollen, and centrifuged once more. This procedure was repeated twice to wash out the acid.

Glycerine–gelatin was prepared by solving 0.5 g phenol in 35 mL distilled water, adding 10 g gelatin, keeping the mixture overnight, and finally adding 30 mL glycerine and stirring the mixture at 48°C for approximately 20 min. Between 100 and 200 µL of glycerine–gelatin were added to each pollen sample, mixed at 48°C, transferred to a clean microscope slide, and covered by a cover slip. After 5 days, the superfluous glycerine–gelatin was removed using a scalpel. The microscope slide was then cleaned with ethanol (70%) and sealed with clear nail polish.

Pollen grains were characterized by morphospecies under a microscope at a magnification of ×40. Where possible, pollen grains were identified to the family or "pollen type" level, following the pollen identification key proposed by Beug (2004).

2.3. Characterizing the amount of sugar added to pollen loads

Pollen loads from bee legs also contain sugars because bees commonly mix pollen collected from flowers with nectar from their crops (Parker 1926; Roulston and Cane 2000; Leonhardt et al. 2007). The

addition of nectar causes an unknown bias to the pollen load's total dry weight (DW) (Roulston and Cane 2000). To account for this bias, we tested for species-, colony-, or source-derived differences in the amount of sugars added to pollen. Pollen loads of single foragers were washed in 400 μL distilled water, kept in the fridge for 1 h, and centrifuged (10 min). The supernatant was transferred to a clean Eppendorf tube, dried at 60°C, and redissolved in 400 μL distilled water. The sugar content of up to 5 μL was measured with a handheld refractometer corrected for temperature (Eclipse, Bellingham & Stanley, Kent, UK) to the nearest 0.5 g/g sucrose equivalent. It was calculated as micromolars of fructose per milligram DW of pollen.

2.4. Amino acid analysis of pollen load mixtures

Free and protein-bound amino acids of pollen load mixtures were analyzed separately by ion exchange chromatography (IEC; Amino Acid Analyzer LC 3000, Eppendorf Biotronik, Hamburg, Germany).

1. To analyze water-soluble amino acids, 0.3–6.4 mg (DW) pollen from mixed samples was extracted with 100 μL water first in an ultrasonic bath (Emmi 20HC, EMAG, Mörfelden-Walldorf, Germany) for 30 min and then in the refrigerator for 60 min. The sample was centrifuged and membrane filtered for 10 min, and the sediment was saved for the subsequent analysis of amino acids in the protein fraction. The supernatant was transferred into a fresh microcentrifuge tube and boiled for 2 min at 100°C. It was then put on ice and cooled down to room temperature before centrifuging it once more for 5 min. To induce precipitation of proteins, 50 μL of the supernatant was extracted with 10 μL of 12.5% 5-sulfosalicylic acid in the refrigerator for 30 min, followed by another 10 min of centrifugation. Finally, 50 μL of the supernatant was mixed with 50 μL thinning buffer in a fresh tube, transferred into a membrane filter (Vecta Spin) and centrifuged (5 min), followed by the IEC analysis.

2. To analyze the amino acid composition of the protein fraction inside the pollen, the sediment was mixed with 200 μL of 6 N HCl$_3$ and boiled for 4 h at 100°C. The sample was cooled down to room temperature and centrifuged (10 min). The supernatant was transferred into a fresh tube. Water was evaporated at 100°C, the sample redissolved in 200 μL of fresh water and centrifuged once more for 10 min. Then, 100 μL of the supernatant was mixed with 20 μL of 12.5% sulfosalicylic acid and extracted in the refrigerator (30 min) before short mixing and centrifuging (10 min). One hundred microliters of the supernatant was mixed with 100 μL sample rarefaction buffer in a fresh microcentrifuge tube, filtered by membrane filtering, and centrifuged for another 5 min. The sample was then transferred into a fresh microcentrifuge tube for further rarefaction with buffer (1:5) and finally analyzed by IEC.

2.5. Statistical analyses

The effects of genera (*Bombus* vs. *Apis*), species, colonies, and pollen sources on variation in sugar content of pollen from single foragers was analyzed using Kruskal–Wallis one-way analysis of variance (ANOVA). Effects of genera, species, and colonies on the variation in total amino acid content from mixed pollen samples were analyzed using generalized linear models (GLMs). GLMs are considered equal to multifactorial ANOVA (Dormann 2008). For this analysis, we excluded one outlier in the data collected for *B. pascuorum* (see Figure 1) that represented more than four times the mean of data points. Data for the total content of water-soluble amino acids were log-transformed to achieve homogeneity of variances. Because of multiple testing ultimately caused by the application of GLMs or multiple Kruskal–Wallis ANOVAs, the level for *p* values below which results were considered significant was set to 0.01 (as suggested by Crawley 2008).

The specific composition of water-soluble and all (free and protein-bound) pollen amino acids in mixed pollen samples was analyzed using an "Adonis" test. "Adonis" represents a multiresponse permutation procedure (library vegan in R, R Foundation for Statistical Computing, version 2009, Vienna, Austria, ISBN: 3-900051-07-0, URL: http://www.R-project.org). It provides a significance (*p* value) and variance explained by intergroup differences (R^2). The test was based on the Bray–Curtis distance matrix of the
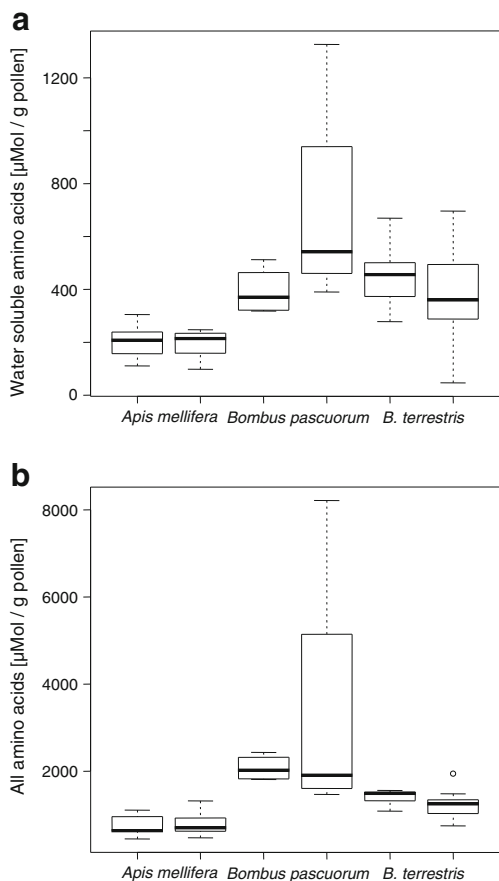
Figure 1. Differences between the six bee colonies (representing three species) in the total amount (in micromolars per gram of pollen) of **a** water-soluble and **b** all (water-soluble and protein-bound) amino acids.

molar proportions of amino acids within each mixed pollen sample (amino acid$_i$ [in micromolars per gram]/total amino acid concentration [in micromolars per gram]) using DW. To further describe the overall degree of chemical partitioning of amino acids across bee species or colonies, we used the quantitative network-level specialization index H_2' (Blüthgen et al. 2006) modified for the analysis of chemical data as described in Leonhardt et al. (2011).

H_2' ranges between 0 (pollen samples from all species/colonies have a similar amino acid composition) and 1 (each species/colony collects pollen with a unique amino acid profile). To assess H_2' for the

amino acid composition of all pollen samples, molar proportions of amino acids were multiplied times 1,000 and rounded to obtain integers, as H_2' can only be calculated for integers. Nonmetrical two-dimensional scaling (NMDS: start configuration: PCoA, 1,000 iterations) was used to produce an ordination figure.

The quantitative network-level specialization index H_2' was further applied to characterize the degree of floral specialization across species/colonies with regard to pollen sources. We used the information provided by both pollen color and pollen grain analysis to test whether pollen color can be used as a proxy of floral partitioning among bee species/colonies. By applying H_2' to both floral partitioning and chemical partitioning (i.e., amino acid composition), we could further observe whether species/colony-specific differences in the range of flowers visited for pollen collection translated into differences in the overall amino acid profile of pollen collected.

3. RESULTS

3.1. Pollen sources and loads

Overall, we characterized 43 different pollen morphospecies. Twelve of these morphospecies could be reliably identified to pollen type according to Beug. Of these, five belonged to the Asteraceae (*Crepis* type (two), *Senecio* type (one), *Echinops* type (one), and *Potentilla* type (one)), three to the Rosaceae (*Rosa* type (two), *Sanguisorba officinalis* type (one)), two to the Plantaginacea (*Plantago lanceolata* and *Plantago major* type), one to the Boraginaceae (*Borago officinalis* type), and one to the Fabaceae (*Vicia* type). Five pollen morphospecies were found in pollen loads of bees more than 10 times, whereas the majority was noted only once (21 pollen morphospecies) or twice (3) (Figure 2).

The three bee species differed in the diversity of pollen types collected, with more pollen morphospecies found in loads of bumblebees than of honeybees (Table I). Honeybees were more flower-constant with regard to pollen morphospecies than were bumblebees (Table I) and had purer pollen loads: 100% of honeybee foragers collected pollen with one pollen mor-
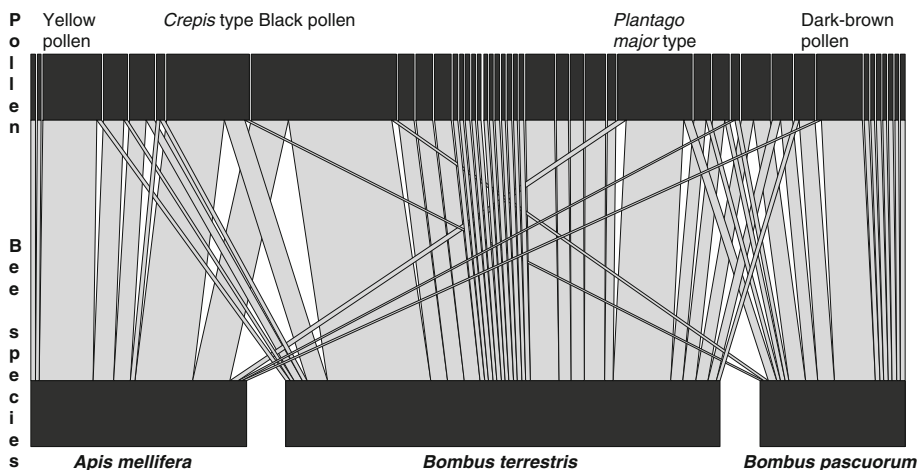
Figure 2. Foraging network, representing three bee species (*bottom*, two genera: honeybees and bumblebees) collecting pollen that comprised 43 different morphospecies (*top*). Block sizes represent overall proportions of pollen types (*top*) or pollen foragers sampled for a given species (*bottom*). The five most frequently collected pollen types (>10 samples per pollen type) are marked.

phospecies representing ≥95% of pollen grains, whereas only 54% of *B. pascuorum* and 76% of *B. terrestris* foragers had comparably pure pollen loads. Moreover, the three species collected largely different spectra of pollen (network-level specialization: $H_2'=0.51$; Figure 2). *B. pascuorum* had the most different pollen spectrum, strongly deviating from both *B. terrestris* ($H_2'=0.57$) and particularly *A. mellifera* ($H_2'=0.72$). *B. terrestris* and honeybee colonies were more similar to each other ($H_2'=0.47$) than the two bumblebee species. The spectrum of pollen collected was, in general, relatively similar among the two colonies of each species (honeybee colonies: $H_2'=0.05$; *B. pascuorum* colonies: $H_2'=0.17$; *B. terrestris* colonies: $H_2'=0.13$).

Similar albeit much less pronounced differences were found when the degree of specialization among bee species was calculated using pollen color instead of pollen morphospecies (all species: $H_2'=0.12$; honeybees vs. *B. pascuorum*: $H_2'=0.22$; honeybees vs. *B. terrestris*: $H_2'=0.12$). Hence, pollen color can be used as a proxy for pollen source partitioning of pollen-collecting bees, but represents a rather conservative approach, as pollen of a given color can comprise between 1 (brown) and 11 (yellow) different pollen morphospecies.

Besides the differences in pollen spectra, the three species also differed in the weight of pollen loads, with *B. terrestris* foragers collecting the heaviest loads (pollen load DW±SD: *B. terrestris*, 12.4±6.7 mg; *B. pascuorum*, 3.8±2.1 mg; *A. mellifera*, 7.0±2.1 mg).

Table I. Pollen foraging behavior with regard to floral resources collected by honeybee and bumblebee colonies.

Species	N	N_P	$N_P>5$
Apis mellifera	42	9	4
Bombus pascuorum	13	18	1
Bombus terrestris	73	30	5

N the number of returning pollen foragers caught per species, N_P the number of pollen morphospecies obtained for each species, $N_P>5$ the number of pollen morphospecies that were found more than five times in all pollen loads of a given species

3.2. Sugar content in pollen

Foragers added between 4.3 and 44.4 μM sugar/mg pollen load. The six colonies differed significantly in the amount of sugar added (Kruskal–Wallis ANOVA: $H=14.2$, $P=0.01$),

but variation was not consistent across bee species ($H=4.7$, $P=0.1$) or genera ($H=2.6$, $P=0.1$) (Figure 3). The bees did not adjust the amount of sugar added to the type of pollen source they foraged on ($H=33.3$, $P=0.3$). Instead, the bees with the heaviest pollen tended to add the least sugar per milligram DW (linear model: $z=-6.1$, $P<0.001$).

3.3. Total protein content and amino acid composition

Variation in total protein content could be best explained by differences between honeybees and bumblebees (Table II), with bumblebees collecting pollen containing more than twice as much protein than pollen of honeybees (water-soluble amino acids (mean±SD): *Bombus* 410±138 μM/g_{DW}, *Apis* 197±61 μM/g_{DW}, t test: $t=5.12$, $P<0.001$; all amino acids (mean±SD): *Bombus* 1,468±388 μM/g_{DW}, *Apis* 778±270 μM/g_{DW}, t test: $t=6.45$, $P<0.001$; Figure 1). The two bumblebee species did not differ in the total amount of water-soluble amino acids (t test: $t=1.08$, $P=0.29$), but in the total amount of all amino acids (t test: $t=4.66$, $P=0.001$). *B. pascuorum* foragers collected pollen with overall twice as much protein (2,723±

2,239 μM/g_{DW}) than found in pollen from *B. terrestris* foragers (1,311±262 μM/g_{DW}).

The composition of amino acids also differed significantly between the two bee genera for both water-soluble (Adonis: $R^2=0.5$, $P=0.001$) and all ($R^2=0.4$, $P=0.001$; Figure 4) amino acids (Table III, IV, and V). However, bumblebees and honeybees had particularly unique amino acid profiles with regard to water-soluble amino acids because chemical partitioning was much more pronounced for water-soluble ($H_2'=0.1$) than for all ($H_2'=0.01$) amino acids (Tables III, IV, and V).

The differences between the two genera were particularly strong for essential amino acids (Tables III and IV): Bumblebees consistently collected pollen with higher proportions of essential amino acids than honeybees (Tables III and IV). Honeybees in turn collected pollen with significantly higher proportions of histidine, proline, and hydroxyproline (Tables III and IV).

4. DISCUSSION

Although all colonies in our study were of floral generalist species and kept at the same habitat with a relatively large resource abundance and heterogeneity, we found striking differences in the species' foraging patterns, which were consistent across colonies. Moreover, differences in foraging patterns translated into differences in nutrient composition of pollen with regard to amino acids.

Not surprisingly, all colonies visited a relatively broad spectrum of plant species for pollen collection. However, bumblebees collected pollen from at least twice as many plant species than honeybees. Bumblebees, particularly *B. pascuorum*, also frequently switched plant species, thereby collecting significant amounts of pollen (grains making up for >20% of pollen loads) from up to three different species during one foraging trip. By contrast, honeybees showed a highly flower-constant foraging behavior and did not switch between plant species during one foraging trip. These observations are in accordance with previous studies that also observed high flower constancy in honeybees
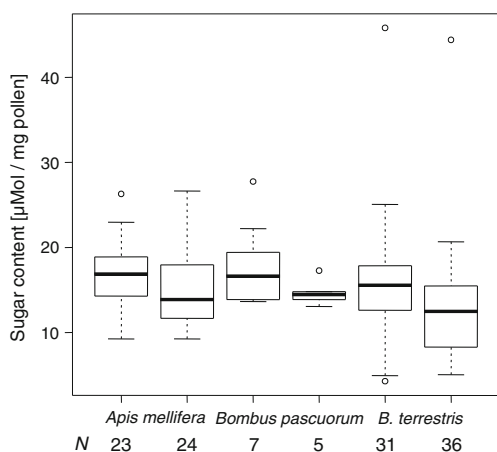

Figure 3. Amount of sugar added by foragers per milligram of pollen (DW) in the six bee colonies observed in our study. N gives the number of pollen samples analyzed per colony.

Table II. Results for GLM, testing for the contribution of genera (*Bombus* and *Apis*), species (*B. pascuorum*, *B. terrestris*, and *A. mellifera*), and colonies (six, two per species) to the overall variation in total protein content of water-soluble amino acids (WS) and all amino acids (All).

	WS			All		
	F	df	p	F	df	p
Genera	19.12	1	<0.001	55.07	1	<0.001
Species	9.88	2	<0.001	41.78	2	<0.001
Colonies	4.45	5	0.003	17.63	5	<0.001

(reviewed by Keller et al. 2005) and a tendency of bumblebees to collect pollen from up to six different plant species during one foraging trip (Kratochwil and Kohl 1988). In addition, our findings reveal that some bumblebee species (e.g., *B. pascuorum*) are more likely to mix pollen than others (e.g., *B. terrestris*). Kratochwil and Kohl (1988) also observed that, at the colony level, *B. terrestris* focused their foraging effort on two to three major (*Tilia cordata* and *Impatiens glandulifera*) and several minor plant species (e.g., *Plantago media*), a strategy that

Figure 4. Similarity in the chemical composition of all (water-soluble and protein-bound) amino acids from mixed pollen loads of honeybee and bumblebee foragers (stress value, 8.17). Ordination figure is based on Bray–Curtis dissimilarities. *Different symbols* represent different species (each symbol represents mixed pollen loads of all foragers of a given species per sampling day).

was also found for 10 bumblebee species in Belgium, England, and The Netherlands (Kleijn and Raemakers 2008) as well as for honeybees (Wille and Wille 1984; Keller et al. 2005). Likewise, the majority of pollen in loads of our *B. terrestris* colonies comprised unidentified black pollen and pollen of the *P. major* type, whereas *B. pascuorum* showed a more even distribution of pollen types in their foraging spectrum.

Notably, the short-tongued *B. terrestris* had a broader spectrum of forage plants than honeybees, but a narrower one than the long-tongued *B. pascuorum*. Kratochwil and Kohl (1988) also reported that *B. pascuorum* visited the largest spectrum of plant species, whereas *B. terrestris* had a rather narrow set of species it foraged on, which was comparable to that of honeybees. The authors related these differences in the spectra of forage plants to morphological differences (i.e., body size and tongue length). They may, however, also arise from differences in the foraging strategies of the two species. Like honeybees, *B. terrestris* foragers prefer large resource patches, whereas *B. pascuorum* foragers dominate small patches (Sowig 1989) and mainly forage on dispersed plants (Teräs 1985). Because bumblebees tend to visit a broader spectrum of plant species than honeybees, they may be potential pollinators for substantially more plant species. However, due to their tendency to mix pollen, bumblebees may also be more likely to transfer heterospecific pollen. Honeybees may in turn transfer less heterospecific pollen and be "better" pollinators for a limited set of plant species.
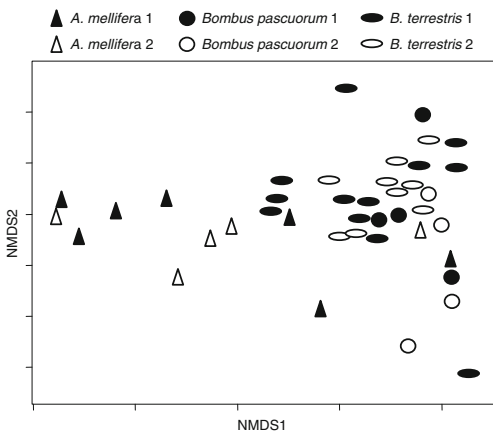
Table III. Proportions of water-soluble amino acids (amino acid [in micromolars per gram]/total amino acid concentration [in micromolars per gram], based on DW) in mixed pollen samples obtained from two *A. mellifera*, two *B. pascuorum*, and two *B. terrestris* colonies between July and September 2010.

Amino acid	Apis mellifera 1	A. mellifera 2	Bombus pascuorum 1	B. pascuorum 2	B. terrestris 1	B. terrestris 2	U	P
1-Methylhistidine	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	198	
3-Methylhistidine	>0.001	>0.001	0.001±0	0.001±0.001	>0.001	>0.001	241.5	*
α-Aminoadipic acid	>0.001	>0.001	0±0.001	0.001±0.001	0.001±0.001	>0.001	207	
α-Aminobutyric acid	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	180	
Alanine	0.055±0.019	0.055±0.015	0.083±0.004	0.082±0.007	0.082±0.006	0.079±0.008	308	***
Ammonium sulfate	0.019±0.005	0.021±0.005	0.039±0.01	0.039±0.006	0.025±0.004	0.045±0.052	301	***
Arginine	*0.02±0.01*	*0.019±0.009*	*0.035±0.018*	*0.033±0.018*	*0.047±0.01*	*0.034±0.012*	*295*	***
Asparagine	0.038±0.031	0.035±0.037	0.038±0.006	0.039±0.003	0.046±0.017	0.044±0.019	238	
Aspartic acid	0.029±0.005	0.027±0.006	0.051±0.008	0.045±0.003	0.039±0.008	0.039±0.012	310	***
β-Aminoisobutyric acid	0.001±0.001	0.001±0.001	>0.001	>0.001	>0.001	>0.001	87	***
β-Alanine	0.002±0	0.002±0.001	>0.001	0±0.001	0.001±0.001	0.001±0.001	26	***
Citrulline	>0.001	>0.001	>0.001	>0.001	0±0.001	0±0.001	186	
Cysteine	0±0.001	0±0.001	>0.001	>0.001	>0.001	>0.001	116	**
Cystathionine	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001	186	
γ-Aminobutyric acid	0.072±0.016	0.077±0.027	0.072±0.01	0.067±0.006	0.069±0.011	0.07±0.01	167	
Glutamine	0.018±0.01	0.014±0.008	0.024±0.004	0.023±0.005	0.026±0.004	0.023±0.006	291	***
Glutamic acid	0.001±0	0.002±0.001	0.002±0.001	0.002±0.001	0.001±0.001	0.002±0.001	153	
Glycine	0.021±0.014	0.02±0.011	0.037±0.009	0.044±0.006	0.046±0.008	0.046±0.01	322	***
Histidine	0.059±0.023	0.054±0.023	0.015±0.002	0.017±0.002	0.021±0.004	0.018±0.003	0	***
Isoleucine	*0.019±0.011*	*0.018±0.009*	*0.054±0.003*	*0.051±0.004*	*0.043±0.007*	*0.04±0.009*	*336*	***
Leucine	*0.041±0.021*	*0.041±0.017*	*0.088±0.007*	*0.08±0.003*	*0.077±0.015*	*0.069±0.015*	*322*	***
Lysine	*0.038±0.017*	*0.036±0.016*	*0.059±0.016*	*0.059±0.013*	*0.063±0.009*	*0.052±0.014*	*297*	***
Methionine	0.015±0.008	0.015±0.006	0.025±0.004	0.026±0.007	0.025±0.005	0.021±0.003	297	***
Hydroxyproline	0.011±0.007	0.012±0.008	>0.001	>0.001	0.002±0.004	0.001±0.003	40	***
Ornithine	>0.001	>0.001	>0.001	>0.001	0±0.001	0.003±0.01	198	
Phosphoethanolamine	>0.001	0±0.001	>0.001	>0.001	>0.001	>0.001	170.5	
Phenylalanine	**0.021±0.009**	**0.021±0.007**	**0.046±0.004**	**0.044±0.007**	**0.038±0.007**	**0.035±0.01**	**324**	***
Proline	0.419±0.187	0.43±0.181	0.113±0.034	0.135±0.05	0.153±0.06	0.187±0.101	52	***

Table III. continued.

Amino acid	Apis mellifera 1	A. mellifera 2	Bombus pascuorum 1	B. pascuorum 2	B. terrestris 1	B. terrestris 2	U	P
Serine	0.035±0.014	0.034±0.014	0.057±0.006	0.056±0.007	0.061±0.006	0.056±0.01	313	***
Taurine	>0.001	>0.001	>0.001	0.001±0.001	>0.001	0±0.001	222	*
Threonine	**0.02±0.011**	**0.02±0.011**	**0.05±0.001**	**0.048±0.003**	**0.043±0.004**	**0.045±0.008**	**337**	***
Tryptophan	0.004±0.002	0.003±0.002	0.007±0.005	0.01±0.003	0.007±0.002	0.007±0.002	309.5	***
Tyrosine	0.013±0.007	0.012±0.005	0.033±0.005	0.034±0.008	0.027±0.005	0.026±0.006	334	***
Valine	*0.028±0.015*	*0.029±0.014*	*0.071±0.004*	*0.067±0.009*	*0.057±0.007*	*0.057±0.017*	**330**	***
N	5	7	4	4	9	12		

Names in bold mark essential amino acids, with the most essential amino acids in italic (for honeybees, according to De Groot 1953)

N the number of mixed samples analyzed for each colony

*P<0.05, **P<0.01, ***P<0.001; significant differences in the proportions of a given amino acid between honeybee and bumblebee colonies (colonies pooled, Wilcoxon matched pairs test)

Interestingly, honeybees and the two bumblebee species visited largely different spectra of flowers for pollen collection, resulting in little overlap in pollen morphospecies among the three species. The species-specific differences in floral spectra visited may partly explain the largely genus-specific amino acid profiles as well as differences in total protein content of pooled pollen loads, with the two bumblebee species collecting pollen of substantially higher quality (comprising more total protein and higher amounts of essential amino acids). Their apparent preference for high-quality pollen (see also Hanley et al. 2008) may represent a tradeoff between the quality and the quantity of forage collected. In contrast to honeybees, bumblebees do not use a dance language or other means of communication to mass recruit foragers to particularly rewarding resource patches. However, unlike honeybees (Keller et al. 2005), bumblebees appear to be able to perceive the quality of pollen. For instance, bumblebees were found to discriminate among *Mimulus guttatus* populations based on pollen quality (Robertson et al. 1999). They were also able to differentiate between experimentally manipulated pollen stores based on high and low pollen protein content (Kitaoka and Nieh 2009). Perception of pollen quality may also explain why differences in amino acid composition in our study were particularly pronounced for water-soluble amino acids which can likely be tasted by foraging bees. Total concentrations of water-soluble and protein-bound amino acids were further significantly correlated (Spearman's rank correlation: $S=4,296$; $P<0.001$), rendering water-soluble amino acids a good indicator of the total amino acid content in pollen of a given flower. Moreover, bumblebees are quite flexible in their foraging behavior and can readily switch from one plant species to another when the latter provides superior food reward with regard to nectar (Heinrich 1976, 1979). This flexibility in behavior, which causes bees to probe other than the current major plant species, may also account for pollen and hence explain why bumblebees were more likely to have mixed pollen loads than were honeybees.

Table IV. Proportions of all amino acids (amino acid [in micromolars per gram]/total amino acid concentration [in micromolars per gram], based on DW) in mixed pollen samples obtained from two *A. mellifera*, two *B. pascuorum*, and two *B. terrestris* colonies between July and September 2010.

Amino acid	Apis mellifera 1	A. mellifera 2	Bombus pascuorum 1	B. pascuorum 2	B. terrestris 1	B. terrestris 2	U	P
1-Methylhistidine	<0.001	<0.001	0.001±0.001	0±0.001	0±0.001	<0.001	240	*
3-Methylhistidine	<0.001	<0.001	0.001±0.001	<0.001	<0.001	<0.001	242.5	*
α-Aminoadipic acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	218	
α-Aminobutyric acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	180	
Alanine	0.083±0.007	0.081±0.006	0.082±0.003	0.085±0.005	0.087±0.003	0.085±0.004	233	
Ammonium sulfate	0.07±0.014	0.068±0.008	0.069±0.018	0.062±0.01	0.058±0.004	0.069±0.02	111	
Arginine	**0.03±0.006**	**0.031±0.004**	**0.04±0.007**	**0.037±0.009**	**0.042±0.003**	**0.037±0.005**	**302**	***
Asparagine	0.01±0.008	0.008±0.006	0.007±0.003	0.01±0.004	0.014±0.007	0.014±0.006	252	*
Aspartic acid	0.086±0.004	0.088±0.008	0.095±0.005	0.091±0.009	0.088±0.004	0.088±0.005	218	
β-Aminoisobutyric acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	96	***
β-Alanine	0.001±0	0.001±0	<0.001	<0.001	<0.001	<0.001	38	***
Citrulline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	192	
Cysteine	0.003±0.001	0.004±0.001	0.004±0	0.004±0.001	0.003±0	0.004±0.001	148	
Cystathionine	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	186	
γ-Aminobutyric acid	0.021±0.004	0.02±0.003	0.016±0.004	0.02±0.006	0.024±0.005	0.024±0.01	214	
Glutamine	0.005±0.003	0.003±0.002	0.005±0.001	0.006±0.003	0.008±0.001	0.007±0.003	291	***
Glutamic acid	0.062±0.006	0.062±0.012	0.07±0.004	0.064±0.01	0.062±0.007	0.063±0.01	198	
Glycine	0.08±0.004	0.08±0.008	0.083±0.004	0.086±0.006	0.08±0.003	0.08±0.006	193	
Histidine	0.031±0.008	0.031±0.008	0.019±0.001	0.02±0.001	0.021±0.001	0.02±0.001	0	***
Isoleucine	*0.024±0.004*	*0.023±0.003*	*0.033±0.003*	*0.035±0.004*	*0.033±0.003*	*0.032±0.004*	*342*	***
Leucine	*0.063±0.008*	*0.064±0.007*	*0.078±0.005*	*0.079±0.001*	*0.078±0.004*	*0.076±0.005*	*326*	***
Lysine	**0.056±0.005**	**0.056±0.005**	**0.053±0.006**	**0.057±0.005**	**0.06±0.003**	**0.055±0.004**	**188**	
Methionine	0.016±0.004	0.017±0.004	0.016±0.006	0.017±0.005	0.019±0.002	0.015±0.004	200	
Hydroxyproline	0.004±0.003	0.003±0.003	0.003±0.002	0.002±0.003	0.001±0.001	0.001±0.002	96	*
Ornithine	<0.001	<0.001	0.001±0.001	<0.001	<0.001	0.001±0.004	210	
Phosphoethanolamine	<0.001	<0.001	0±0.001	<0.001	<0.001	<0.001	177	

Table IV. continued.

Amino acid	Apis mellifera 1	A. mellifera 2	Bombus pascuorum 1	B. pascuorum 2	B. terrestris 1	B. terrestris 2	U	P
Phenylalanine	**0.031±0.003**	**0.035±0.006**	**0.054±0.01**	**0.043±0.009**	**0.038±0.006**	**0.037±0.009**	**278**	**
Proline	0.16±0.062	0.162±0.068	0.063±0.011	0.074±0.016	0.084±0.016	0.094±0.032	56	***
Phosphorserine	<0.001	<0.001	0.001±0.001	<0.001	<0.001	<0.001	234	
Serine	0.064±0.007	0.065±0.008	0.073±0.002	0.071±0.003	0.072±0.002	0.07±0.003	274	**
Taurine	<0.001	<0.001	0±0.001	<0.001	<0.001	<0.001	234	
Threonine	**0.044±0.007**	**0.043±0.004**	**0.054±0.002**	**0.055±0.003**	**0.052±0.001**	**0.054±0.002**	**347**	***
Tryptophan	0.001±0	0.001±0.001	0.001±0.001	0.003±0.002	0.002±0.001	0.002±0.001	302.5	***
Tyrosine	0.021±0.004	0.02±0.003	0.028±0.003	0.031±0.004	0.025±0.002	0.025±0.002	315	***
Valine	*0.034±0.005*	*0.036±0.003*	*0.046±0.002*	*0.047±0.006*	*0.045±0.003*	*0.046±0.005*	**345**	***
N	5	7	4	4	9	12		

Names in bold mark essential amino acids, with the most essential amino acids in italic (for honeybees, according to De Groot 1953)

N the number of mixed samples analyzed for each colony

*P<0.05, **P<0.01, ***P<0.001; significant differences in the proportions of a given amino acid between honeybee and bumblebee colonies (colonies pooled, significance values as in Table III)

Table V. Test results for differences among amino acid profiles of different bee genera (HB and BB), species (HB, BB1, and BB2), and colonies (_1 and _2).

AA	Level tested	Groups tested	H_2'	R^2	P
WS	Genus	HB vs. BB	0.09	0.52	*<0.001*
All	Genus	HB vs. BB	0.01	0.35	*<0.001*
WS	Species	BB1 vs. BB2	<0.01	0.08	0.07
All	Species	BB1 vs. BB2	<0.01	0.10	0.02
WS	Colonies	HB_1 vs. HB_2	<0.01	<0.01	0.89
All	Colonies	HB_1 vs. HB_2	<0.01	0.02	0.87
WS	Colonies	All BB colonies	–	0.12	0.32
All	Colonies	All BB colonies	–	0.18	0.05
WS	Colonies	BB1_1 vs. BB1_2	<0.01	–	–
All	Colonies	BB1_1 vs. BB1_2	<0.01	–	–
WS	Colonies	BB2_1 vs. BB2_2	<0.01	–	–
All	Colonies	BB2_1 vs. BB2_2	<0.01	–	–

Italicized *p* values indicate *p* values significant after correction for multiple testing (using sequential Bonferroni)

AA amino acids, *WS* water-soluble, *HB A. mellifera*, *BB1 B. pascuorum*, *BB2 B. terrestris*

The significantly higher quality of pollen collected by bumblebees compared to pollen collected by honeybees may thus be a consequence of the bees' ability to perceive and judge food quality and optimize their foraging behavior accordingly. Whereas honeybees may be more likely to simply follow the hint of dancers returning from a large patch of any plant species that makes the recruitment of a large troop of foragers worthwhile, bumblebees may count on the decisions of individual foragers that aim to optimize the quality of resources they collect. This ability is all the more impressive as both bumblebee species collect high-quality pollen, although they employ quite different foraging strategies (with *B. terrestris* preferring large patches of flowering plants and *B. pascuorum* preferring scattered plant sources; Sowig 1989).

The finding that *B. pascuorum* and *B. terrestris* collect pollen from more plant species and of higher quality than honeybees and that they are more likely to mix pollen from different plant species suggests that the two *Bombus* species are as much floral generalists as honeybees, but specifically rely on those plant species that provide pollen of high quality (e.g., Fabaceae: Hanley et al. 2008).

They may hence be more prone to suffer from human-induced decline in resource abundance and diversity if human impact disproportionally decreased the abundance of plants with high-quality pollen (e.g., Fabaceae: Goulson et al. 2005) and increased the abundance of plants with pollen of lower quality (e.g., crop plant species). Also, colony size was found to be positively correlated with foraging range (Walther-Hellwig and Frankl 2000; Darvill et al. 2004). Hence, smaller- and medium-sized colonies (e.g., *B. pascuorum* colonies) may be more likely to be affected by reduced resource abundance at local landscape scales (Westphal et al. 2006). Further studies comprising more species (particularly the less common ones) and colonies are needed to better understand how resource abundance and diversity affect the colony fitness of social bees via resource quality. Besides the total protein and amino acid content, pollen quality can also relate to the composition of minerals and lipids. Minerals were found to vary among plant species (reviewed by Keller et al. 2005), resulting in different mineral levels of bee-collected pollen (Herbert and Miller-Ihli 1987). It is, however, unclear if and how differences in pollen

mineral composition can be perceived by bees and if they affect the health of colonies. It is also unclear if pollen varies in lipid content and whether lipid composition of pollen affects the foraging behavior and health of bees.

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Semblable mais cependant différente: la récolte du pollen chez les colonies d'abeilles et celles de bourdons.

Pollinisateurs généralistes / acides aminés / ressources florales / qualité du pollen

Gleich, und doch verschieden. Wie Honigbienenvölker und Hummelvölker Pollen sammeln.

generalistische Bestäuber / Aminosäuren / florale Ressourcen

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