



Pollen nutrients better explain bumblebee colony development than pollen diversity

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Abstract. 1. Bumblebees are valuable pollinators of numerous wild and cultivated plants. They can forage on variable pollen resources. As some pollen species lack particular nutrients or have global low nutritional quality, it has been suggested that bumblebees mix their pollen incomes to ensure a global balanced diet. The hypothesis that a mixed pollen diet better supports bumblebee colony development than a single pollen diet has been poorly explored.

2. We compared the impact of mono-, di- and trifloral diets on microcolony development of *Bombus terrestris* using three pollen resources with different nutrient contents (*Cytisus scoparius*, *Erica* sp. and *Sorbus aucuparia*) as well as their mixes. Nine parameters (e.g. pollen efficacy: total weight of larvae/total weight of pollen collection) were used to compare the microcolony performances. Moreover, we measured the influence of the pollen diversity and nutritional composition on relevant parameters.

3. We showed that microcolonies can potentially better develop on mixed pollen diets, but single pollen diet can also be as good as mixed pollen diet. Moreover, the sterol concentration appeared as a key factor to establish the impact of a pollen diet on the bumblebee colony development.

4. This study reveals that diverse pollen diet does not necessary equate with good colony development and supports the importance of selecting floral resources by considering their nutrient contents for bee conservation management.

Key words. Amino acids, bumblebee, colony, diet, nutrient, sterol.

Introduction

The conservation of pollinators has been a major issue during the last decades (Goulson *et al.*, 2015). This raises concerns not only for the diversity of pollinator species (e.g. 20 000 bee species, the main group of pollinators; Michener, 2007) but also for over 87% of flowering plants that are animal pollinated (Ollerton *et al.*, 2011). In fact, pollinator conservation is essential for flowering plant reproduction and in a broader sense for the stability of

ecosystems, including agricultural ecosystems (Potts *et al.*, 2010).

Bumblebees (*Bombus* spp., Apidae, Hymenoptera) are abundant pollinators in temperate ecosystems including that of crops (Corbet, 1991; Dias *et al.*, 1999). Unfortunately, bumblebees are drastically declining in Europe (Carvalheiro *et al.*, 2013; Nieto *et al.*, 2014), North America (Cameron *et al.*, 2011) and Asia (Williams & Osborne, 2009), which compromises the provided ecosystem services. A variety of specific or global threats have been described such as habitat destruction, decrease of both floral resources quantity and diversity, pesticides, diseases and climate change (Inouye, 2007; Williams *et al.*, 2008; Goulson *et al.*, 2015; Kerr *et al.*, 2015). The relative

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importance of these factors and their potential interactions are still under debate (Potts *et al.*, 2010). More experimental studies are needed to better evaluate the threats to bumblebees and to propose mitigation strategies (Nieto *et al.*, 2014; Vaudo *et al.*, 2015). Herein, we developed a study addressing the importance of mixed pollen diet quality for bumblebee colony growth. This question is of crucial interest in simplified ecosystems such as agricultural areas, where monocultures provide from a restricted set to a single pollen type.

Bumblebee diets depend entirely on floral resources (i.e. pollen and nectar) foraged from a wide range of plant species from different families (e.g. Kleijn & Raemakers, 2008). The nutritional content of pollen (i.e. proteins, amino acids, sterols and vitamins) may differ greatly from one plant species to another (e.g. Roulston *et al.*, 2000; Di Pasquale *et al.*, 2013; Vanderplanck *et al.*, 2014). Unfortunately, we know little about the different nutritional requirements of bee species (Vaudo *et al.*, 2016). The variations in chemical compositions appear to influence colony development (Tasei & Aupinel, 2008a; Vanderplanck *et al.*, 2014; Kamper *et al.*, 2016). For example, bumblebee larval development is slower when fed with pollen containing a lower amino acid content (Moerman *et al.*, 2015). Bumblebees are not helpless when facing this variability as they are apparently able to discriminate flowers with different pollen chemistry. They preferentially forage on species providing pollen with higher protein and essential amino acid content (Hanley *et al.*, 2008; Leonhardt & Blüthgen, 2012; Ruedenauer *et al.*, 2015; Somme *et al.*, 2015; Kriesell *et al.*, 2016) and with a precise range of protein/lipid ratio (Vaudo *et al.*, 2016). In addition, they can display pollen mixing behaviour at individual level (e.g. Somme *et al.*, 2015). This strategy ensures optimal nutritional requirements because mixed pollen diets (i.e. pollen diets including more than one plant species) can mitigate the lack of nutrients or the presence of toxic chemicals in a monofloral diet (Arnold *et al.*, 2014; Eckhardt *et al.*, 2014). Therefore, we may expect that mixed pollen diets are more suitable than monofloral diets. This has been demonstrated in the immune system of the honeybee (*Apis mellifera* L.) (Alaux *et al.*, 2010) and their tolerance of parasites (Di Pasquale *et al.*, 2013). As far as we know, the relative quality of mixed pollen diets for bumblebee colony development compared to monofloral diets remains poorly understood (Baloglu & Gurel, 2015) while this question is crucial for designing mitigation strategies for bumblebee conservation (i.e. selection in the diversity and quality of managed host plants). In this study we compared the development of *Bombus terrestris* L. microcolonies (i.e. queenless colonies) fed monofloral diets from *Cytisus scoparius*, *Erica* sp. or *Sorbus aucuparia* to the development of microcolonies fed their di- and trifloral equivalent diets (i.e. double and triple combinations of these three plant species). Additionally, we tested the relation between pollen nutrient profile (e.g. total amino acids, essential amino acids and sterols) and developmental parameters. Based on the generalist

behaviour of *B. terrestris* and the pollen preferences of bumblebees, we hypothesised that a mix of pollen diets from resources of high nutritive value will positively impact bumblebee colony development.

Material and methods

Pollen sources

We considered pollen diets previously described from wild populations of bumblebees in heathlands and bogs (Belgium) based on palynological analyses of worker pollen loads (Mayer *et al.*, 2012). The authors had identified three major pollen resources: *C. scoparius* (Fabaceae), *S. aucuparia* (Rosaceae) and *Vaccinium uliginosum* (Ericaceae). We additionally considered pollen from *Salix caprea* and *Cistus* sp. as positive and negative references, respectively, to monitor microcolony development during the experiment (see below). These two pollen diets were previously, respectively, described as favourable and unfavourable resources for *B. terrestris* colony growth (Vanderplanck *et al.*, 2014). The sterol content (composition and concentration) and amino acid concentrations of these five pollen resources differ but they all contain the full spectrum of essential amino acids (Table 1; Vanderplanck *et al.*, 2014) and support colony development (Génissel *et al.*, 2002; Tasei & Aupinel, 2008a; Vanderplanck *et al.*, 2014; Moerman *et al.*, 2015).

Diets were prepared using honeybee pollen loads supplied by hives with pollen traps in areas where the target plants were in bloom and dominant. Unfortunately, it was impossible to collect pollen from *V. uliginosum*, which is unattractive to worker honeybees. A commercial blend (i.e. 'Pollenenergie' blend) with dominant *Erica* sp. pollen was used as an alternative. As *Erica* and *Vaccinium* belong to the same plant family, they were expected to display similar pollen protein content (Roulston *et al.*, 2000). In the same manner, no significant difference has been detected in the sterol composition between the two genera (the three major sterol compounds were the same: δ^5 -avenastrol, δ^7 -avenasterol and β -sitosterol; M. R. Moerman, unpublished data).

Worker honeybees forage on pollen from various resources but each worker individually specialises usually in one pollen resource (e.g. Leonhardt & Blüthgen, 2012), making a monofloral pollen load. As the target pollen species may be differentiated based on their colour, we stored five single pollen batches by removing all non-target pollen loads based on this criterion. We double-checked the uniqueness of the plant species composition of the final five pollen blends by analysing the pollen grain morphology under a light microscope (Leitz at $\times 400$ magnification) to compare with a reference library (Moore *et al.*, 1991) and floral pollen collected from the targeted plants. Lastly, di- and trifloral pollen batches were produced by mixing the single pollen batches as

Table 1. Chemical content of the three monofloral diets and the two controls ($n = 3$ per group).

Diets	Total amino acid concentration (mg/g in lyophilised matter)	Essential amino acid concentration (mg/g in lyophilised matter)	Total sterol concentration (mg/g in lyophilised matter)	The most abundant sterols
<i>Cistus</i> sp.	135.3 ± 5.9 (D)	59.4 ± 2.3 (D)	2.47 (A)	24Methylencholesterol/campesterol β -sitosterol δ 5-avenasterol
<i>Cytisus scoparius</i>	300.7 ± 23.4 (A)	146.2 ± 11.5 (A)	2.46 ± 1.1 (A)	24Methylencholesterol/campesterol β -sitosterol δ 5-avenasterol
<i>Erica</i> sp.*	135.3 ± 8.6 (D)	66.8 ± 4.1 (D)	7.36 ± 2.17 (AB)	β -sitosterol δ 5-avenasterol δ 7-avenasterol
<i>Salix caprea</i>	186.5 ± 9.6 (C)	96.1 ± 5.4 (C)	5.33 ± 1.05 (AB)	24Methylencholesterol/campesterol β -sitosterol δ 5-avenasterol
<i>Sorbus aucuparia</i>	235.5 ± 4.6 (B)	111.6 ± 2.5 (B)	9.64 ± 1.68 (B)	24Methylencholesterol/campesterol β -sitosterol δ 5-avenasterol

Different bold, uppercase letters indicate significant difference among diets (*post hoc* tests, $P < 0.05$) (according to Vanderplanck *et al.*, 2014).

*Identified as *Calluna* sp. in Vanderplanck *et al.* (2014).

follows: (i) *S. aucuparia/Erica* sp. (50/50, w/w), (ii) *S. aucuparia/C. scoparius* (50/50, w/w), (iii) *Erica* sp./*C. scoparius* (50/50, w/w) and (iv) *S. aucuparia/Erica* sp./*C. scoparius* (30/30/30, w/w/w).

The nine pollen diets (two controls, three mono-, three di- and one trifloral) were prepared using the same ratio of mass pollen with inverted sugar syrup (Biogluc; Biobest, Westerlo, Belgium) (90% and 10% w/w respectively) to obtain pollen pastes that are easier to manipulate than fresh pollen. These pollen pastes were weighed and stored at -20 °C before being provided to the microcolonies.

Using previous results of Vanderplanck *et al.* (2014) (Table 1), the total sterol concentration, the total amino acid concentration and the essential amino acid concentration were calculated for the mixed pollen diets as the mean of single pollen diet contents.

Microcolony rearing

Seven microcolonies per diet were reared in a dark room at 26–28 °C and 65% relative humidity. One microcolony consisted of four 2-day-old worker *B. terrestris* from the same colony (Biobest bvba, Westerlo, Belgium) placed in plastic boxes (10 × 16 × 16 cm) and fed *ad libitum* with inverted sugar syrup (Biogluc) and pollen paste during a 12-day period following the first egg laid. Bioassays of queenless microcolonies of *B. terrestris* have been approved for testing the nutritive value of pollen diets and can be extrapolated to a queenright colony (Tasei & Aupinel, 2008b). The pollen paste was changed every 2 days to avoid a potential decrease in the nutritive value and to calculate the pollen collection by the workers (difference of paste weight before and after the 2 days).

Preliminary test did not reveal any significant evaporation of the syrup from pollen paste in 2 days in experimental conditions.

Performance on diet was assessed using several parameters adapted from Tasei and Aupinel (2008a): (i) number and fresh weight of isolated larvae (last stage before pupation), (ii) number of ejected larvae, (iii) pollen collection (i.e. amount of pollen consumed and stored) (fresh matter), (iv) worker mortality and (v) syrup collection (i.e. amount of syrup consumed and stored). Using these parameters, pollen efficacy was calculated by dividing the total weight of larvae by the total weight of pollen collection. This parameter was used for the first time by Tasei and Aupinel (2008a) and later by Vanderplanck *et al.* (2014) to evaluate the diet quality of bumblebees.

To estimate the impact of mixed pollen diet (i.e. pollen mixing) on *B. terrestris* microcolony development, we additionally determined the theoretical values of developmental parameters (i.e. pollen efficacy, weight of isolated larvae and total larvae number) for the di- and trifloral diets as the mean of their monofloral components (e.g. mean results of *C. scoparius* pollen diet and *S. aucuparia* monofloral diets as the theoretical results of the *C. scoparius/S. aucuparia* difloral diet). We compared these theoretical values to the observed values.

Statistical analysis

The rearing parameters between the different diets were compared using one-way analyses of variance (one-way ANOVAS) after checking for normality (Shapiro test on residuals) and homoscedasticity (Bartlett test) assumptions. When violation(s) occurred even after log-

transformation, data were rank-transformed (i.e. non-parametric model). *Post hoc* tests (multiple pairwise comparisons) were conducted when a significant difference was detected by the one-way ANOVAs. In addition, the reliability of the statistical outputs was checked by performing power tests for unbalanced design. To visually assess the differences in criteria quality among the five tested pollens, we constructed side-by-side boxplots. Lastly, the theoretical and observed developmental parameters for the di- and trifloral diets were compared using Student *t*-tests (after checking for normality with the Shapiro test) to underline the potential impact of pollen mixing.

Correlation test was performed between the different colony performance parameters to establish non-correlating variables. The results were visualised on a correlogram (*corrplot* package and function). Moreover, linear regressions were performed with non-correlated colony parameters and nutritional value for each diets (e.g. total amino acids, essential amino acids and total sterol concentration).

All data visualisation and analyses were performed in R version 2.2.1 with Sciviews R Console (version 0.9.2) (R Core Team, 2013).

Results

Development of microcolonies

Microcolonies fed with the positive control diet (i.e. *Salix* sp.) produced a significantly higher total weight of isolated larvae (2.78 ± 0.91 g) than microcolonies fed with the negative control diet (i.e. *Cistus* sp.) (0.93 ± 0.6 g) ($t = -5.2$, $P < 0.001$). Additionally, pollen efficacy (i.e. total weight of isolated larvae/total weight of pollen collection) of the *Salix* monofloral diet (0.77 ± 0.2) was significantly higher than that of the *Cistus* monofloral diet (0.24 ± 0.17) ($t = -5.9$, $P < 0.001$).

The number of larvae did not depend on the diet (isolated larvae, $F_{8,50} = 1.58$, $P = 0.156$, power = 0.63; ejected larvae, rank-transformed, $F_{8,50} = 1.14$, $P = 0.355$, power = 0.47). By contrast, the total weight of isolated larvae was significantly different depending on the diet ($F_{8,50} = 10.37$, $P < 0.001$, power = 1). Microcolonies fed with the *Erica* sp. monofloral diet (2.52 ± 0.55 g) produced the lowest weight of isolated larvae (0.93 ± 0.6 g), whereas microcolonies reared on the *C. scoparius* monofloral diet produced the highest weight of isolated larvae (3.76 ± 0.74 g). Multiple pairwise comparisons revealed that the detected differences were not related to the pollen diversity of the diet (Table S1). Worker mortality was observed in only two microcolonies fed the *C. scoparius* monofloral diet (i.e. one of the four workers was found dead in each of these two microcolonies) (rank-transformed data, $F_{8,50} = 2.81$, $P = 0.012$, power = 0.90) (Table S1).

There were significant differences among the diets in terms of pollen and syrup collection (pollen, $F_{8,50} = 6.66$,

$P < 0.001$, power = 1; syrup, $F_{8,50} = 6.25$; $P < 0.001$, power = 1). The main differences, however, did not depend on the pollen diversity of the diets, as the monofloral diets showed similar values to the mixed diets (Table S1). Workers in microcolonies fed with the *Erica* sp. monofloral diet collected the highest amount of syrup (64.17 ± 3.58 ml) while the lowest amount was collected by workers in microcolonies fed with the *C. scoparius* monofloral diet (53.67 ± 6.57 ml).

There was a significant difference in pollen efficacy between the diets ($F_{8,50} = 24.45$, $P < 0.001$, power = 1): the *Erica* sp. monofloral diet displayed the lowest efficacy (0.65 ± 0.1) and the *C. scoparius/S. aucuparia* difloral diet displayed the highest efficacy (1.16 ± 0.18) (Fig. 1).

Impact of mixed pollen diet

In microcolonies fed with difloral diets, the observed developmental results were not significantly different from the theoretical results, with the exception being the weight of isolated larvae in microcolonies fed with the *S. aucuparia/Erica* sp. difloral diet ($t = -3.4$, $P = 0.01$) with value lower than expected. Interestingly, the observed efficacy of the trifloral diet (*C. scoparius/Erica* sp./*S. aucuparia*) (1.07 ± 0.08) was significantly higher than the theoretical efficacy (0.9) ($t = 4.8$, $P = 0.003$) (Table 2).

Correlation between rearing parameters

Some of the seven rearing parameters were correlated: pollen efficacy was positively correlated with the number of larvae ($r_p = 0.56$; $P = 0.025$) and the weight of larvae ($r_p = 0.87$; $P < 0.001$) but negatively correlated with the syrup collection ($r_p = -0.55$; $P = 0.014$) (Fig 2, Table S2). Moreover, syrup collection was negatively correlated with the weight of larvae ($r_p = -0.61$; $P = 0.003$) and the number of larvae ($r_p = -0.24$; $P = 0.049$). As predictable, the weight of larvae and the number of larvae were positively correlated ($r_p = 0.71$; $P = 0.006$).

Influence of nutrient contents on the colony development

We performed linear regressions between the non-correlate rearing parameters (total pollen collection, worker mortality, number of ejected larvae and pollen efficacy) and the concentration of three pollen nutrients (amino acids, essential amino acids and sterols) (Table 3 and Table S2). The pollen efficacy was highly influenced (R-squared = 0.74) by the nutrient content ($F_{3,55} = 53.6$; $P < 0.001$) and especially by sterol concentration ($P < 0.001$) and the essential amino acid concentration ($P = 0.005$). To a lesser extent (R-squared = 0.38), the total pollen collection was influenced by the nutrient content ($F_{3,55} = 11.1$; $P < 0.001$) and by the total sterol concentration ($P = 0.011$). In the same way (R-

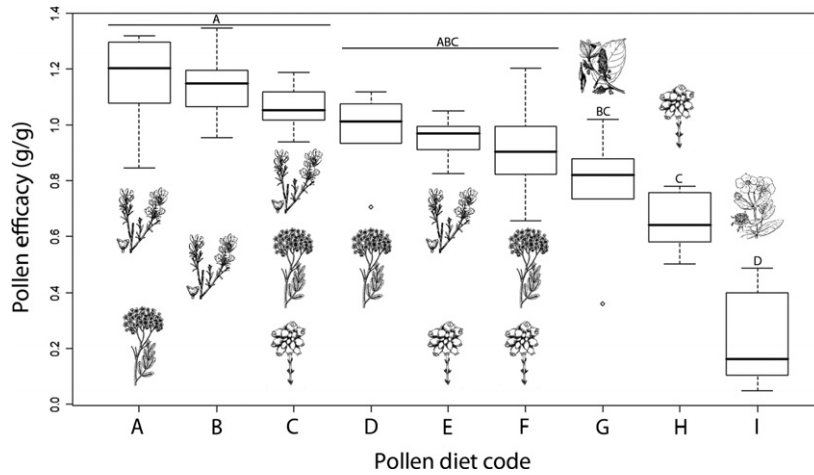


Fig. 1. Pollen efficacy of microcolonies fed different pollen diets. Uppercase letters indicate significant difference between diets (*post hoc* tests, $P < 0.05$). Medians are given in box plots and outliers are marked with circles. Pollen diet codes: A = *C. scoparius*/*S. aucuparia* difloral diet, B = *C. scoparius* monofloral diet, C = *C. scoparius*/*S. aucuparia*/*Erica* sp. trifloral diet, D = *Sorbus aucuparia* diet, E = *C. scoparius*/*Erica* sp. difloral diet, F = *S. aucuparia*/*Erica* sp. difloral diet, G = *Salix* sp. monofloral diet, H = *Erica* sp. monofloral diet and I = *Cistus* sp. monofloral diet.

Table 2. Comparison of observed (obs., $n = 7$) and theoretical (th.) developmental parameters of microcolonies fed on di- and trifloral diets.

Parameters	<i>C. scoparius</i> / <i>S. aucuparia</i>			<i>C. scoparius</i> / <i>Erica</i> sp.			<i>S. aucuparia</i> / <i>Erica</i> sp.			<i>C. scoparius</i> / <i>S. aucuparia</i> / <i>Erica</i> sp.		
	Obs.	Th.	Stat.	Obs.	Th.	Stat.	Obs.	Th.	Stat.	Obs.	Th.	Stat.
Pollen efficacy	1.2 ± 0.8	1.1	$t = 0.8$ $P = 0.44$	0.9 ± 0.1	0.9	$t = 1.8$ $P = 0.12$	0.9 ± 0.2	0.8	$t = 1.7$ $P = 0.15$	1.1 ± 0.1	0.9	$t = 4.8$ $P = 0.003$
Number of isolated larvae	13.8 ± 4.7	11.2	$t = 1.37$ $P = 0.23$	10.3 ± 3.1	11.3	$t = -0.8$ $P = 0.45$	10.1 ± 2.7	9.4	$t = 0.68$ $P = 0.52$	10.4 ± 3.3	10.7	$t = -0.5$ $P = 0.63$
Weight of isolated larvae (g)	3.4 ± 0.6	3.4	$t = 0.18$ $P = 0.87$	3.1 ± 0.6	3.1	$t = -0.03$ $P = 0.98$	2.3 ± 0.3	2.8	$t = -3.4$ $P = 0.01$	3.1 ± 0.4	3.1	$t = 0.08$ $P = 0.94$

Theoretical values were considered the mean value of the observed monofloral diets. Student *t*-tests (Stat.) were conducted on pollen efficacy (total weight of larvae/total weight of pollen collection), number of isolated larvae and weight of isolated larvae. Observed data are presented as mean ± SD. Significant results are indicated in bold.

squared = 0.18), the worker mortality was influenced by the chemical content ($F_{3,55} = 4.07$; $P < 0.011$) and particularly the sterol concentration ($P = 0.034$).

Discussion

Mixed pollen diet and nutrient content of pollen

Our results confirm that the pollen of Fabaceae (*C. scoparius*) and Rosaceae (*Sorbus aucuparia*) constitute suitable resources for *B. terrestris* (Tasei & Aupinel, 2008a; Vanderplanck *et al.*, 2014). Moreover, the impact of pollen nutrients (e.g. sterol concentration) on the colony development was also corroborated by our bioassays (Tasei & Aupinel, 2008a; Vanderplanck *et al.*, 2014;

Moerman *et al.*, 2015). In previous work, the role played by amino acids content on bumblebee colony growth and foraging behaviour was underlined (Moerman *et al.*, 2015; Kriesell *et al.*, 2016). Linear regressions (Table 3) suggest that the essential amino acid concentration is correlated with the pollen efficacy and consequently to the bumblebee colony development. Moreover, correlation test reveals that pollen efficacy is positively correlated with the number and the weight of larvae (Fig. 2, Table S2); it would be predictable that nutrient contents influence the larval growth. Because they play an essential role in hormone synthesis, gene expression and cell membrane function (Behmer & Nest, 2003), sterols are probably key nutrients for the growth of the bumblebee larvae. Protein/lipid ratio was pointed by Vaudo *et al.* (2016) to drive bee pollen-foraging behaviour. Our results suggest that both

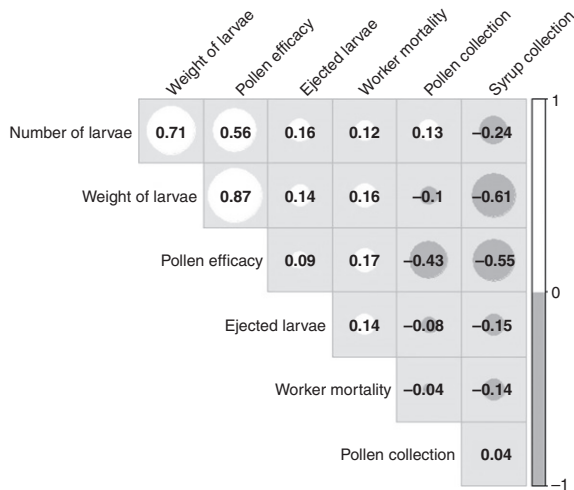


Fig. 2. Correlogram showing results of the correlation test performed on development colony parameters. Diameter of the circle is proportional to the correlation index.

components (e.g. sterols concentration and essential amino acid concentration) probably have major influence on bumblebee colony development.

Our findings demonstrate that microcolonies of *B. terrestris* can potentially develop significantly better when fed di- or trifloral diets compared to those fed monofloral diets depending of the nutrient contents of the pollen found in the diet. In fact, microcolonies provided with monofloral diets containing suitable nutritive values (e.g. *C. scoparius* and *S. aucuparia*) produced larger larvae than microcolonies fed difloral diets containing unsuitable nutritive content (i.e. mixes containing *Erica* sp. pollen). These bioassays on *B. terrestris* reflect those performed on honeybees (Alaux *et al.*, 2010; Di Pasquale *et al.*, 2013), corroborating the fact that di- and trifloral diets are not generally better than monofloral diets.

Overall, the effect of pollen mixing on colony development seems to be related to the composition of each diet and to the originality of the combination. The pollen efficacy of the trifloral pollen diet was higher than expected (Table 2). This result is probably linked to more suitable nutrient combination (e.g. amino acid and sterol profiles) of the three pollen included in the diet (see Table 1). The weight of larvae from microcolonies fed on difloral pollen diets of *S. aucuparia* and *Erica* sp. was lower than

expected (Table 2). The concentration of nutrients is not particularly low (see Table 1) but the specific presence of the δ^7 -avenasterol in *Erica* sp. pollen potentially explains the negative effect of the combination (Rasmont *et al.*, 2005).

Diet quality and floral choices of bumblebees

Different studies have shown that host plant specialisation allows the workers to forage more efficiently on the resource (e.g. Lavery & Plowright, 1988). Based on this ecological advantage, evolution to host plant specialisation should be promoted in bumblebees. Most of bumblebee species forage on multiple hosts (i.e. generalist behaviour) even at individual level (e.g. Brian, 1952; Free, 1970; Kleijn & Raemakers, 2008; Leonhardt & Blüthgen, 2012; Somme *et al.*, 2015). It is then expected that generalist behaviour provides some alternative evolutionary advantages for bumblebees.

One foreseen advantage of diet mixing is dilution of the potential toxicity of a monofloral diet (Eckhardt *et al.*, 2014). Our bioassays corroborate this hypothesis because the pollen of *C. scoparius* induced worker mortality when provided as monofloral diet, probably because of the quinolizidine alkaloids (Sundararajan & Koduru, 2014), but not when blended in a di- or trifloral diet (i.e. alkaloid dilution). Such mixing behaviour could be a global strategy for generalist bees as has been described for many herbivorous insects (e.g. Bernays & Bright, 1993).

Resource quality is not the only factor that drives floral choices in bumblebees. Although different studies have shown that bumblebees tend to forage on resources with high amino acid content (Leonhardt & Blüthgen, 2012; Somme *et al.*, 2015; Kriesell *et al.*, 2016), the main foraged resource in the Upper Ardennes (i.e. *V. uliginosum*) belongs to Ericaceae (Mayer *et al.*, 2012), whose pollen has a relatively less concentrated amino acid content and is on the whole less suitable for bumblebees (Vanderplanck *et al.*, 2014). This unexpected floral choice is probably led by the density of ericaceous species in this ecosystem (i.e. heathlands) because worker bumblebees increase their visits to dense floral patches (Waser, 1986; Kunin & Iwasa, 1996; Kamper *et al.*, 2016).

The floral choices of bumblebees are therefore a complex process involving multiple inputs probably including

Table 3. Linear regressions with non-correlating colony parameters and nutritional value for each diets.

Parameters	<i>F</i> stat.	<i>P</i> -value	R-squared	Sterols (<i>P</i> -value)	AA (<i>P</i> -value)	EAA (<i>P</i> -value)
Total pollen collection (g)	$F_{3,55} = 11.1$	< 0.001	0.38	0.011	0.811	0.432
Worker mortality	$F_{3,55} = 4.07$	0.011	0.18	0.034	0.976	0.787
Ejected larvae	$F_{3,55} = 1.46$	0.23	0.07	0.54	0.311	0.411
Pollen efficacy (g/g)	$F_{3,55} = 53.6$	< 0.001	0.74	< 0.001	0.127	0.005

Significant results are indicated in bold.

the accessibility and availability of resources (e.g. floral morphology and population density) as well as their chemical composition (e.g. primary and secondary metabolites).

Bumblebee food resources management

The optimisation of mitigation strategies to conserve plant and pollinator diversities is one of the key challenges for ecologists in the 21st century (Mayer *et al.*, 2011). As low diversity of wild floral resources was noted as a major cause of bee decline (Biesmeijer *et al.*, 2006; Goulson *et al.*, 2015), one of the main actions in many recent bee conservation strategies is promoting a high diversity of resources via agro-environmental schemes (Dicks *et al.*, 2013) but with only low consideration for the floral resource quality (Dicks *et al.*, 2015; Vaudo *et al.*, 2015). Field studies have questioned this strategy by showing that pollinator diversity is not always positively influenced by the plant diversity proposed in agro-environment schemes (Wood *et al.*, 2015). Our results additionally reveal that, more than pollen diversity, the pollen chemical composition is a key factor for bumblebee development and should be considered with great attention in the near future for their conservation. In this manner, the efficiency of conservation strategies might be related to the target plant species included in agro-environment measures than to their diversity (i.e. number of species). We suggest that nutrient content of resources should be a priority investigation in habitat management for bumblebees to sparingly select plant resources. Moreover, because different insect species are known to have different nutritional targets (Behmer, 2009), more investigations are needed concerning other bumblebee species than *B. terrestris*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12213:

Table S1. Parameters of microcolonies performance for the nine diets ($n = 7$ microcolonies). Data are presented as

mean \pm SD. Uppercase letters indicate significant differences among diets (post-hoc tests, $P < 0.05$).

Table S2. Parameters of microcolonies performance for the nine diets ($n = 7$ microcolonies). Data are presented as mean \pm SD. Uppercase letters indicate significant differences among diets (post-hoc tests, $P < 0.05$).

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