Bumblebees depend on ericaceous species to survive in temperate heathlands

LAURA MOQUET,[1] MARYSE VANDERPLANCK,[2] ROMAIN MOERMAN,[2,3] MURIEL QUINET,[1] NATHALIE ROGER,[2] DENIS MICHEZ[2] and ANNE-LAURE JACQUEMART[1]   [1]Research Group Genetics, Reproduction, Populations, Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium, [2]Laboratory of Zoology, Research Institute for Biosciences, Université de Mons, Mons, Belgium and [3]Evolutionary Biology and Ecology, Université Libre de Bruxelles, Brussels, Belgium

Abstract. 1. Bumblebees are the predominant wild pollinators for many plant species in temperate regions. A bumblebee colony requires pollen and nectar throughout its lifetime, but degraded and fragmented habitats may have gaps in the temporal and spatial continuity of floral resources.

2. Heathlands are open biotopes that provide favourable habitat for bumblebees like *Bombus jonellus*, a declining species in Belgium. In heathlands, ericaceous species are the main plants that provide pollen and nectar for bumblebees. Although the nectar composition of ericaceous species has been previously studied, data on pollen composition remain scarce.

3. We examined bumblebee diets (composition of their pollen loads) in Belgian heathlands over the course of a colony lifetime to assess the fidelity of bumblebees for ericaceous species. We compared nutritional values by investigating the chemical composition (amino acids, polypeptides and sterols) of the pollen of the ericaceous and dominant non-ericaceous species present in pollen loads. No relationship was detected between the abundance of a particular plant species in bumblebee loads and its pollen composition.

4. The successive flowering periods and the nutritional quality of pollen of ericaceous species offer valuable resources for bumblebees. Ericaceous species represent a large part of bumblebee diets in heathlands, especially in early spring and late summer when the diversity of other flowering species was low.

5. Bumblebee pollen loads also contained non-ericaceous flowering species that grow outside heathlands. Thus, land planning must incorporate conservation strategies for the different elements of the landscape matrix, including heathlands, peatlands, meadows and margins.

Key words. *Bombus jonellus*, bumblebee diet, nectar, pollen.

Introduction

Pollinators play an important role in most terrestrial ecosystems, contributing to the reproduction of about 78% of plant species in temperate regions (Ollerton *et al.*,

Correspondence: Laura moquet, Research group Genetics, Reproduction, Populations, Earth and Life Institute, Université catholique de Louvain, Croix-du-Sud 2, Box L7.05.14, B-1348 Louvain-la-Neuve, Belgium. E-mail: laura.moquet@uclouvain.be

2011). Among pollinators, bumblebees (Hymenoptera, Apidae, *Bombus*) are the predominant wild bee pollinators for many wildflowers and crops (Corbet *et al.*, 1991; Osborne & Williams, 1996; Baldock *et al.*, 2015). Their conservation has therefore received major attention in recent years (Goulson *et al.*, 2015), even as alarming evidence about their worldwide decline has accumulated (Rasmont *et al.*, 1993; Kosior *et al.*, 2007; Grixti *et al.*, 2009; Cameron *et al.*, 2011; Carvalheiro *et al.*, 2013; Nieto *et al.*, 2015). This population decline involves multiple causes, but one primary factor concerns modifications

of the landscape matrix (Hendrickx *et al.*, 2007; Goulson *et al.*, 2010; Bennett & Isaacs, 2014), including destruction, fragmentation and degradation of habitats (Rathcke & Jules, 1993; Fahrig, 2003; Harris & Johnson, 2004). These alterations of habitats through modifications of landscape use (urbanisation, agricultural practices) cause shifts or decreases in the quantity and/or quality of floral resources (i.e. pollen and nectar) available for bumblebees (Biesmeijer *et al.*, 2006; Kleijn & Raemakers, 2008; Goulson *et al.*, 2015).

Bumblebees are entirely dependent on floral resources for survival. Nectar consists of a blend of sugars and represents the major energy source for adult bumblebees; pollen consists mostly of proteins and lipids (Roulston & Cane, 2000) and represents the major nutrient source for larval development (Génissel *et al.*, 2002; Kitaoka & Nieh, 2008). Among lipids, insects use phytosterols in several key metabolic pathways such as the synthesis of molting (Behmer & Nes, 2003) and ovariole maturation hormones (Bloch *et al.*, 2000). Also, protein concentration directly affects reproduction, growth, immunocompetence and longevity of insects (Gilbert, 1981; Smeets & Duchateau, 2003; Alaux *et al.*, 2010). Protein content alone, however, is not sufficient to define the nutritional value of pollen for bees, as it also depends on the amino acid composition (Cook *et al.*, 2003; Nicolson, 2011; Moerman *et al.*, 2015). As bumblebees cannot synthesize essential amino acids and phytosterols *de novo*, they rely exclusively on pollen for these essential compounds (de Groot, 1953; Behmer & Nes, 2003).

Bumblebees do not store much food in their nests, therefore, they require continuous access to floral resources throughout the lifetime of the colony, from early spring to late summer (Goulson *et al.*, 2005). In spring, in the nest-founding phase, the newly emerged queens eat both nectar and pollen (Prŷs-Jones & Corbet, 1987), using the nutrients in pollen to complete ovary development and start egg production (Génissel *et al.*, 2002; Human *et al.*, 2007). In the colony development phase, after the workers begin to eclose, the queen continues to lay eggs while the workers take care of the brood and collect pollen and nectar. In the mating phase, the colony produces males and new queens (Duchateau & Velthuis, 1988; Alaux *et al.*, 2005). New queens feed on pollen and nectar, which provide energy stores to allow them to undergo diapause (Prŷs-Jones & Corbet, 1987). Different bumblebee species have different timings of their colony life cycle exist, but generally, emergence occurs between March and May and the colony cycle extends from 14 to 25 weeks (Goodwin, 1995; Benton, 2006). Species with short colony cycles, such as *B. pratorum* and *B. jonellus*, are more dependent on high-quality food to quickly rear larvae (Goulson & Darvill, 2004).

Disruption in the continuity of flowering resources can threaten bumblebee life cycles, for example, in the fragmented habitats currently typical of heathlands. Heathlands are open, semi-natural habitats dominated by ericaceous species (the heather family) and were widespread in Europe during the 18th and 19th centuries due to human activities like grazing of livestock (Gimingham, 1960). In the late 20th century only a small part of the European heathlands remains (Rebane *et al.*, 1997); most of these biotopes have been converted to agricultural uses or forested areas like spruce plantations (Aerts & Heil, 1993; Webb, 1998). The remaining heathlands constitute a refuge for many rare species and are subject to European legislation to protect their biodiversity (Birds Directive 79/409/EEC – European Commission, 1979 and Habitats Directive 92/43/EEC – European Commission, 1992). Heathlands support populations of several specialist pollinators and provide major habitat for *Bombus jonellus* Kirby, a declining bumblebee species especially threatened in Belgium (Rasmont *et al.*, 1993). The destruction of heathlands, as well as their low floral diversity (Forup *et al.*, 2007), suggest that the availability of floral resources for bumblebees may have temporal or spatial gaps that could threaten bumblebee survival.

Species belonging to the Ericaceae are the main entomophilous plant family in the heathlands. Most ericaceous species have poricidal anthers (Hermann & Palser, 2000; Jacquemart, 2003), which release pollen only in response to vibration, termed 'buzz pollination' (Buchmann, 1983). Buzz pollination is energetically costly but potentially advantageous in terms of nutritional quality or reduced competition, as only a few insects, such as bumblebees, can pollinate these plants. In recent years, many studies have examined how pollen composition affects the attraction of pollinators (Roulston & Cane, 2000; Aupinel *et al.*, 2001; Cook *et al.*, 2003; Human *et al.*, 2007; Vanderplanck *et al.*, 2014b), but most studies have focused on species with easily accessible pollen, rather than buzz-pollinated species (Roulston *et al.*, 2000; Vanderplanck *et al.*, 2014a). Roulston *et al.* (2000) observed that buzz-pollinated taxa contain pollen particularly rich in proteins. Other studies, however, showed differences in pollen composition among ericaceous species (Vanderplanck *et al.*, 2014b; Moquet *et al.*, 2015).

In this study, we used pollen loads to analyse the diet of bumblebee individuals visiting flowers of ericaceous species throughout the lifetime of the bumblebee colony. By assessing the quantity and quality of pollen and nectar produced by the species present in the bees' diet, we determined whether these plants provide valuable resources for bumblebee colonies. We addressed three questions: (i) Do bumblebees use the floral resources of all ericaceous species throughout the colony lifetime? We hypothesised that ericaceous species form a major part of the diet of bumblebees in heathlands, (ii) Do the pollen and nectar of ericaceous plants constitute valuable resources? Bumblebees discriminate between resources according to their quality; therefore, we expected ericaceous species to provide nectar and pollen of high nutritional quality, and (iii) Is the nutritional content of ericaceous pollen linked to bumblebee fidelity, i.e., the constancy of an individual to a particular flowering species? We hypothesised that low-quality pollen would induce bees to collect pollen from other species.

Methods

Bumblebee observations

Studied sites and periods of observations. Observations and sampling were carried out in 10 wet heathland sites located in the Upper Ardenne, Belgium (Table 1). These sites contained 7 of the 8 Belgian Ericaceae: *Andromeda polifolia* L., *Calluna vulgaris* L. Hull, *Erica tetralix* L., *Vaccinium myrtillus* L., *V. oxycoccos* L., *V. uliginosum* L. and *V. vitis-idaea* L. We observed the bumblebee visitors to the four most abundant ericaceous species in heathlands, namely, in the order of flowering, *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix* and *Calluna vulgaris* (named hereafter 'target plant species'), for three successive years (2013–2015). Observations were performed on *V. myrtillus* between 14 May 2013 and 5 June 2013 and between 9 April 2014 and 16 May 2014. Observations on *V. vitis-idaea*, the second main flowering species, were performed between 14 June 2013 and 2 June 2013, between 19 May 2014 and 11 June 2014 and between 2 June 2015 and 25 June 2015. Observations on *E. tetralix* were performed between 9 July 2013 and 19 July 2013, between 24 June 2014 and 6 August 2014 and between 25 June 2015 and 20 August 2015. Observations on the last flowering species, *C. vulgaris*, were performed between 9 August 2013 and 21 August 2013, between 5 August 2014 and 24 August 2014 and between 24 July 2015 and 26 August 2015.

Observations were separated along the season based on bumblebee colony phases. We defined three phases of bumblebee colony lifetime and delimited them depending on the dominant caste present during observations. In this way, we delimited (i) the nest-founding phase, which corresponded to observations on *V. myrtillus* when bumblebee queens were abundant; (ii) the colony development phase, which corresponded to observations on *V. vitis-idaea* and *E. tetralix* when workers were dominant; and finally, (iii) the mating phase, which corresponded to observations on *C. vulgaris* when newly emerged queens and males were dominant.

Bumblebee diversity. For each of the four successive target plant species, observations took place on sunny, windless days in 6 or more of the 10 sites, depending on the availability of the target plant species. Bumblebee visitors were recorded during one to four entire days per site, per year, and for each target plant species (Table 1). Surveys were carried out on 10 m² plots of continuous shrub cover representative of the population for 20 minutes each hour between 9.00 a.m. and 6.00 p.m. (Mayer *et al.*, 2012). A total of 125 days or 354 h of observations were conducted over the 3 years. A total of 2980 bumblebees were collected with an insect net and were identified. We noted sex and caste of individuals, and whether they carried corbicular pollen loads. We released them on the plot immediately after the 20-min period of observations. Due to their high morphological similarity, individuals of *B. terrestris*, *B. lucorum*, *B. cryptarum* and *B. magnus* were pooled into one operational taxonomic unit (*B. terrestris* OTU) and *B. hortorum* and *B. jonellus* into another OTU (*B. hortorum* OTU, Terzo & Rasmont, 2007). At the end of the observation periods, several individuals of each *Bombus* morphotype were killed (102 individuals in total) with ethyl acetate for identification in laboratory based on an identification key (Rasmont & Terzo, 2010).

Pollen loads. In 2013 and 2014, during the 20-min periods of observations, bumblebees with pollen loads visiting target plant species were immobilised in a bee-marking cage and one of the two pollen loads was carefully removed using a toothpick. In the laboratory, pollen loads were weighed to check the influence of load weight on taxa diversity. The samples were then acetolysed (Erdtman, 1960, modified) for palynological analyses by light microscopy (Leitz Wetzlar). Pollen grain identification was based on a reference collection from the Université catholique de Louvain, an identification key (Reille, 1992) and a comprehensive list of flowering plants in the studied sites. A total of 343 pollen loads was analysed. To detect all the pollen species present and to accurately estimate the proportions of the different pollen species, a minimum of 400 randomly chosen pollen grains were identified per vortexed pollen load sample, as in other recent studies (Mayer *et al.*, 2012; Moisan-Deserres *et al.*, 2014).

Floral diversity. To facilitate and increase accuracy of pollen identification in bumblebee loads, we recorded all other flowering entomophilous plant species that cover more than 1 m². Floral diversity surveys were carried out just before bumblebee observations within a radius of 200 m around the studied plots.

Assessment of pollen and nectar quality

Floral pollen collection. At the peak of flowering, that is, between early April and late August, stems (30–200 depending on plant species, number of flowers per stem and the quantity of pollen per flower) of the four target plant species and of the main non-ericaceous species detected in bumblebee pollen loads were harvested from a minimum of five individual plants per site in six sites. Only ericaceous species with population size and density allowing enough pollen collection were studied. Stems were kept in tap water for one night at room temperature (approximately 20 °C) in the laboratory. The next morning, stamens were extracted from newly open flowers and dried at room temperature for 12 h after which pollen was removed using a sieve (Sieve 3″, Brass–Stainless, Full Height, 80 μm). Because the majority of the ericaceous species studied (*V. myrtillus*, *V. uliginosum* and *E. tetralix*) had poricidal anthers, pollen was collected from the flowers by vibration. A small vibrator was built out of a 5-mm rod, which was slightly inclined and attached to a small handle (Sami Yunus; Institute of Condensed Matter

Table 1. Location and size of studied sites situated in the Upper Ardenne, Belgium.

Sites		Grande Fange	Wé des Pourceaux	Pisserotte	Crépale	Fanges aux Mochettes	Grand Passage	Pouhon	Robièfa	Nazieufà	Sacrawé
Coordinates		50°14′40″N 5°46′45″E	50°14′42″N 5°44′57	50°13′13″N 5°47′54″E	50°16′40″N 5°44′07″E	50°13′21″N 5°40′55″E	50°13′44″N 5°45′46″E	50°14′32″N 5°41′51″E	50°15′27″N 5°42′10″E	50°15′05″N 5°43′09″E	50°14′33″N 5°45′46″E
Localities		Vielsalm	Vielsalm	Gouvy	Lierneux	La Roche-en-Ardenne	Houffalize	Manhay	Manhay	Manhay	Vielsalm
Natural reserve area (ha)		282	NA	206	30	71	165	57	34	37	282
Heathland area (ha)		0.31	1.15	1.6	7.63	9.58	10.91	18.55	25.38	35.87	40.67
Days of observation of *V. myrtillus*	2013	1	–	2	–	2	3	–	2	–	2
	2014	1	–	1	–	3	2	–	3	–	3
Days of observation of *V. vitis-idaea*	2013	1	–	1	–	–	–	–	–	–	1
	2014	2	1	2	–	–	2	–	–	2	3
	2015	2	2	2	–	–	2	–	–	2	2
Days of observation of *E. tetralix*	2013	–	–	–	–	2	3	2	2	–	2
	2014	–	–	–	3	3	2	3	3	–	4
	2015	2	–	–	2	2	2	4	4	–	2
Days of observation of *C. vulgaris*	2013	2	–	2	–	1	1	1	–	–	3
	2014	1	–	1	–	–	–	–	1	–	2
	2015	2	–	2	–	2	–	2	2	–	2

The years of observation of bumblebee visits are detailed for each of four target plant species with number of observation days. Sites are arranged by increasing area of heathland.

and Nanosciences, Université catholique de Louvain). Rod rotations could be modulated to produce vibrations between 200 and 800 Hz. Pure pollen samples were pooled by species to reach at least 200 mg (the amount required for chemical analyses), lyophilised and stored at −20 °C.

Chemical composition of pollen. The polypeptide content (molecular weight >10 000 Da) was quantified from 5 mg dry pollen in triplicate for each species following the method described by Vanderplanck *et al.* (2014a). The quantification of total polypeptide content was performed using the standard curve of the BCA Protein Assay Kit (Pierce, Thermo Scientific), at the University of Mons (Mons, Belgium).

The amino acid content was quantified from 3 mg dry pollen in triplicate for each species following the method described in Vanderplanck *et al.* (2014a). Total amino acids were measured separately by ion exchange chromatography and post-column ninhydrin derivatisation (Biochrom 20 plus amino acid analyser) at the University of Liège (Gembloux, Belgium). Only tryptophan was omitted because its isolation requires separate alkaline hydrolysis from an additional amount of sample. Essential amino acids for bumblebees were assumed to be the same as those identified for honeybees (i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine; de Groot, 1953).

Phytosterol content was quantified from 15 mg of dry pollen for each species following the method described in Vanderplanck *et al.* (2011). The total phytosterol content was determined considering all quantifiable peaks of sterols that eluted between cholesterol and betulin (internal standard) at the University of Liège (Gembloux, Belgium). Identifications were made by comparing the relative retention times with those of a sunflower oil reference. Due to technical problems, we were unable to provide total sterol content for *Salix* x *multinervis*.

Pollen volume per flower. Because pollen grains vary widely in size, pollen volume provides a better indicator of the nutrient storage capacity than the number of pollen grains (Buchmann & O'rourke, 1991). For the main plant species present in the bumblebee pollen loads, we collected flower buds from 10 individuals in two different populations. Buds were fixed in FAA (ethanol 70% : formaldehyde 35% : acetic acid, 8:1:1) before counting. To extract pollen, anthers were dissected, dropped into a known volume of Alexander's red solution (300–3200 µl, depending on pollen quantity), crushed with a plastic rod, sonicated and vortexed. A 5-µl drop of pollen suspension was deposited on slides and all pollen grains were counted. Pollen dimensions were measured with Motic Images Plus (Version 2.0) software from pictures taken with a Moticam2000 camera using light microscopy (Nikon Eclipse E400) at a magnification of 450×. Pollen volumes (V) were calculated using the measured longitudinal (l) and equatorial (e) lengths according to the formula $V = 1/6\pi l^3$

for spheroidal grains and $V = 1/6e^2 l$ for ellipsoidal grains (Buchmann & O'rourke, 1991; Rasheed & Harder, 1997).

Nectar volume and composition. We sampled nectar at the peak of flowering for each target plant species. In 2014, on at least 6 days per plant species with optimal weather conditions (no rain in 24 h), glass capillary tubes of 0.5 µl or 1 µl (Hirschmann Laborgeräte, Eberstadt, Germany) were used to collect nectar from a minimum of 10 freshly opened flowers from five bushes. Depending on the species, between 60 (*Vaccinium* spp.) and 720 flowers (*C. vulgaris*) were sampled. The nectar volume was estimated by measuring the length of the nectar column in the capillary tube. In the laboratory, nectar tubes were pooled by species and site to reach at least 0.5 mg and stored at −80 °C before analyses. Sugar composition was determined by gas chromatography with a PerkinElmer Autosystem XL equipped with a split injector (1/20) and helium as carrier gas (flow of 1 ml/min). The injector and detector temperatures were maintained at 250 and 350 °C respectively. Sugar (i.e. sucrose, glucose and fructose) analyses for nectar composition were performed at the Centre Apicole de Recherche et d'Information (CARI, Louvain-la-Neuve, Belgium).

Statistical analyses

All analyses were conducted in R (R Development Core Team, 2013) and, if not indicated otherwise, data are presented as mean ± standard deviation.

For each target plant and bumblebee species, we excluded loads from the statistical analyses when $n < 5$. We also excluded cuckoo bumblebees (subgenus *Psithyrus*) due to their different foraging behaviour (i.e. no worker caste and no pollen collection, Prŷs-Jones & Corbet, 1987). To determine whether the proportion of bumblebee individuals collecting pollen, proportion of pollen derived from visited plant species and the number of plant species in pollen loads differed per bumblebee species and per plant species visited, we performed generalised linear mixed models (GLMM) analysis with year of observations and studied sites as random factors and with year nested within studied sites. If random effects were not significant, we preferentially conducted GLM analysis. We used a binomial error distribution for proportion of bumblebee individuals collecting pollen, quasi-binomial for proportion of pollen of visited plant species in bumblebee loads and quasi-Poisson for number of taxa in bumblebee loads. To verify the absence of correlation between the plant taxa number in the pollen loads, the weight of pollen loads and the number of flowering plant species around the studied plots, we performed Spearman's rank correlation tests.

Because assumptions for parametric tests (i.e. normality and homoscedasticity) were not respected, we used Kruskal–Wallis tests to compare polypeptide, amino acid, essential amino acid and phytosterol contents among

plant species. If an overall significant difference was detected, pairwise comparisons using Tukey and Kramer (Nemenyi) tests were conducted. To test differences in amino acid and sterol composition among pollen types, we performed a permutational multivariate analysis of variance (perMANOVA) using the Euclidean distance matrix and 999 permutations ('adonis' command, R-package vegan). Prior to this perMANOVA, the multivariate homogeneity of within-group covariance matrices was verified using the 'betadisper' function. Both similarities and dissimilarities in chemical compositions among the different pollen sources were visually assessed using principal component analysis (PCA). In PCA calculated on the percentage of total of amino acids in pollen, we added the ideal balance determined by de Groot (1953) for honeybees excluding tryptophan (arginine 11.5%, histidine 5.2%, isoleucine 14.6%, leucine 16. 7%, lysine 11.5%, methionine 5.2%, phenylalanine 9.4%, threonine 11.5% and valine 14.6%), as an illustrative individual.

Results

Fidelity for ericaceous floral resources throughout the colony lifetime

Foraging behaviour on ericaceous plant species. Bumblebees were observed on the ericaceous plants species throughout the colony lifetime from April on *V. myrtillus* to August on *C. vulgaris*. Beside the subgenus *Psithyrus*, six different operational taxonomic units (OTUs) were observed using ericaceous resources: *Bombus hortorum* OTU (in which 22/27 individuals were identified as *B. jonellus* Kirby and the remaining 5 as *B. hortorum* L.), *B. lapidarius* L., *B. terrestris* L., *B. hypnorum* L., *B. pascuorum* Scopoli and *B. pratorum* L. Bumblebee species differed significantly in their foraging behaviour (GLMM; $\chi_5 = 51.6$; $P < 0.001$; Fig. 1). Although *B. jonellus* collected pollen on all ericaceous species throughout the colony lifetime (except on *C. vulgaris*), *B. pascuorum* visited ericaceous species mainly for nectar and collected significantly less pollen than other species (GLMM; $P < 0.05$). *Bombus hypnorum* individuals displayed intermediate foraging behaviour, as they foraged *V. vitis-idaea* pollen in heathlands, but did not forage on other ericaceous species.

The proportion of bumblebees collecting pollen differed significantly among the target plant species (GLMM; $\chi_4 = 186.5$; $P < 0.001$; Fig. 1) from 63% on *V. vitis-idaea* during colony development (mainly for pollen) to 13% on *C. vulgaris* at the end of colony lifetime (mainly for nectar). On average, 22% of bumblebees caught on *V. myrtillus* and *E. tetralix* carried pollen loads.

Pollen collection. The analysis of both the proportion of the target ericaceous species and the number of other plant species in bumblebee pollen loads revealed that bumblebees collecting pollen showed a high fidelity for
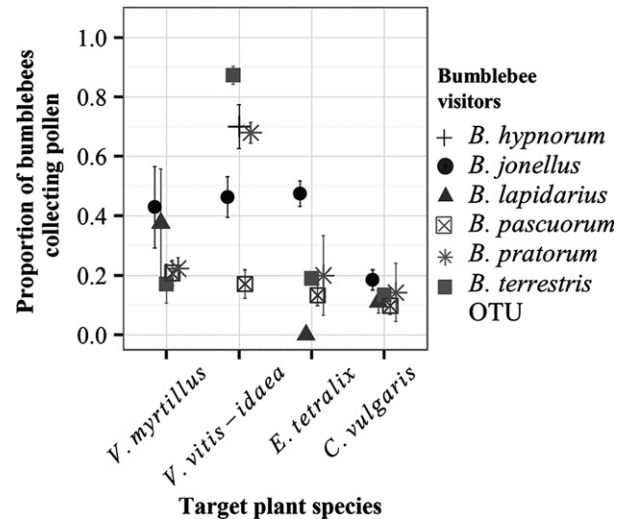

Fig. 1. Proportions of bumblebees collecting pollen on the four target plant species in the 10 studied sites. Data are shown as mean ± SE. *Bombus terrestris* OTU (operational taxonomic units) refers to *B. terrestris*, *B. lucorum*, *B. cryptarum* and *B. magnus*.

ericaceous species. The proportion of pollen of target plant species (Fig. 2a) was significantly lower for bumblebees caught on *V. myrtillus* (0.56 ± 0.45; GLMM; $t_{309} = -3.7$; $P < 0.001$), *V. vitis-idaea* (0.79 ± 0.33; GLMM; $t_{309} = -3.2$; $P = 0.02$) and *E. tetralix* (0.65 ± 0.40; GLMM; $t_{308} = -3.8$; $P < 0.001$) than for bumblebees caught on *C. vulgaris* in late summer (0.86 ± 0.26). Among bumblebee species, *B. jonellus* individuals had a higher proportion of pollen from ericaceous species in their loads (0.80 ± 0.32) compared with *B. terrestris* OTU (0.70 ± 0.38; GLMM; $t_{308} = -3.15$; $P = 0.002$) and *B. pascuorum* (0.55 ± 0.43; GLMM; $t_{308} = -2.97$; $P = 0.003$).

We did not find any correlation between the mean number of plant taxa in the pollen loads and the weight of pollen loads ($r^2 = -0.10$; $t_{74} = -0.93$; $P = 0.35$) or the number of flowering plant species around the studied plots ($r^2 = -0.05$; $t_{92} = 0.46$; $P = 0.65$). The number of different plant taxa (Fig. 2b) was higher in loads of bumblebees caught during colony development on *V. vitis-idaea* (GLM; $t_{308} = 2.5$; $P = 0.01$) and *E. tetralix* (GLM; $t_{315} = 2.6$; $P = 0.009$) than in loads of bumblebees caught during mating phase on *C. vulgaris*. Among bumblebees, *B. jonellus* individuals had significantly lower plant taxa diversity in their pollen loads than *B. terrestris* OTU individuals (GLM; $t_{308} = 2.4$; $P = 0.03$).

We observed that 63% of pollen loads were monospecific (i.e. >95% conspecific pollen grains) and 83% of them contained only pollen of the target ericaceous species. In addition to the target plant species, bumblebees collected pollen on trees and shrubs like *Salix* spp. and *Sambucus racemosa* during the nest-founding phase, on *Cytisus scoparius*, *Narthecium*
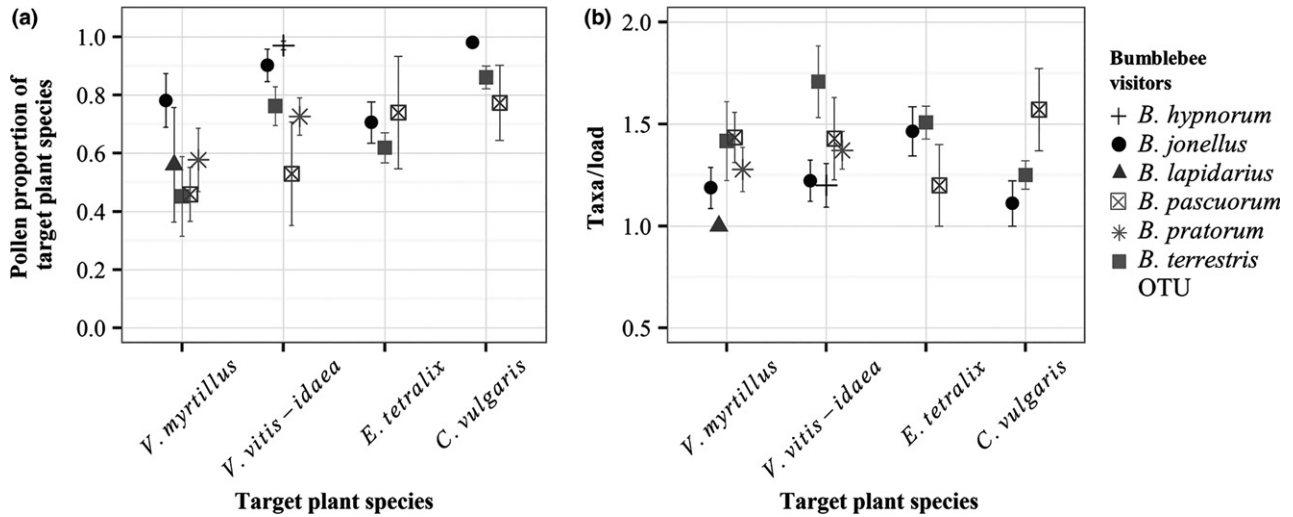
Fig. 2. Fidelity of the bumblebees estimated by pollen load analysis. (a) Proportions of pollen of the target ericaceous plant species and (b) number of the different plant taxa in pollen loads, for each bumblebee species caught on target plant species in the 10 studied sites. Data are shown as mean ± SE. *Bombus terrestris* OTU (operational taxonomic units) refers to *B. terrestris*, *B. lucorum*, *B. cryptarum* and *B. magnus*.

ossifragum and *Rubus* spp. during colony development and on *Filipendula ulmaria* in the last phase of colony life (Fig. 3). Plant species in the diet of *B. jonellus* originated mainly from heathlands, peatlands (e.g. *Narthecium ossifragum*, *Salix* spp.) and heathland edges (e.g. *Cytisus scoparius*, *Rubus* spp., *Sambucus racemosa*). By contrast, the other bumblebee species foraged pollen on plant species growing in nearby biotopes like meadows and woods (e.g. *Malus* spp., *Vicia* spp., *Acer* spp. or *Hypericum* spp.; Fig. 3). The presence of plant species in pollen loads coincided with the peak of flowering of each plant (Table 2).

Bumblebee colony phases	Nest-founding phase							Colony development																Mating phase		
Target plant species	*V. myrtillus*							*V. vitis-idaea*								*E. tetralix*								*C. vulgaris*		
Plant species	Vaccinium spp. (E)	Salix spp. (Sal)	Sambucus racemosa (A)	Malus spp. (R)	Cytisus scoparius (F)	Vicia spp. (F)	Crataegus spp. (R)	Vaccinium spp. (E)	Cytisus scoparius (F)	Rubus spp. (R)	Sambucus racemosa (A)	Acer spp. (Sap)	Sorbus spp. (R)	Hypericum spp. (H)	Crataegus spp. (R)	Erica tetralix (E)	Narthecium ossifragum (N)	Rubus spp. (R)	Trifolium repens (F)	Lotus spp. (F)	Trifolium pratense (F)	Tilia spp. (M)	Filipendula ulmaria (R)	Calluna vulgaris (E)	Filipendula ulmaria (R)	Erica tetralix (E)
B. jonellus	78.0	8.6	12.9					89.8	3.2						5.2	70.2	11.4	9.0	5.5		3.3			98.1		1.6
B. lapidarius	55.9	14.1		29.2																						
B. terrestris OTU	44.8	25.9	22.9	3.0				99.8	0.2							59.0	12.2	12.2	4.0	4.4	2.8	1.7	1.6	86.8	8.2	2.7
B. hypnorum								75.2	6.3	7.9		2.7	3.6													
B. pascuorum	46.0	27.5	11.5			9.2	4.1	52.0	47.0							73.2	19.5	6.8						87.8		9.9
B. pratorum	57.3	22.7			19.2		1.2	72.6	12.7	1.2	5.0	4.9	1.7	0.6												
Total	56.1	20.9	10.0	5.0	2.8	2.8	1.2	72.0	13.4	3.2	2.4	2.3	1.7	1.5	1.4	62.7	12.1	10.8	4.1	3.0	2.8	1.1	1.0	88.4	6.3	3.2

Fig. 3. Percentage of pollen grains of the different plant species present in diet of bumblebee individuals caught on *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix* and *Calluna vulgaris* in the 10 studied sites. Only pollen of plant species with total percentage > 1 and *Bombus* species with sample > 5 pollen loads are shown. Plant species indicated in bold were studied by pollen chemical analyses. *Bombus terrestris* OTU (operational taxonomic unit) refers to *B. terrestris*, *B. lucorum*, *B. cryptarum* and *B. magnus*. Plant family indicated in brackets. A: Adoxaceae; E: Ericaceae; F: Fabaceae; H: Hypericaceae; M: Malvaceae; N: Nartheciaceae; R: Rocaceae; Sal: Salicaceae; Sap: Sapindaceae.

Table 2. Flowering periods of the different plant species (adapted from Lambinon & Verloove, 2012) and number and volume (mm^3) of pollen grains per flower (n = 10).

	Flowering periods	Bumblebee colony phases	Pollen grains/flower	Volume/flower (mm^3)
Salix x *multinervis*	March–May	Nest-founding phase	13921 ± 4830	2.46 ± 0.41
Malus pumila	April–May	Nest-founding phase	7125 ± 1258	0.24 ± 0.04
Vaccinium myrtillus	April–June	Nest-founding phase	51668 ± 2902*	0.69 ± 0.16
Cytisus scoparius	May–July	Colony development	24781 ± 19353	0.36 ± 0.28
Sorbus aucuparia	May–July	Colony development	67273 ± 11673	0.7 ± 0.12
Vaccinium uliginosum	May–July	Colony development	17760 ± 693*	0.26 ± 0.04
Vaccinium vitis-idaea	May–June	Colony development	46794 ± 1818*	0.56 ± 0.09
Rubus spp.	June–July	Colony development	117593 ± 16534	0.96 ± 0.14
Erica tetralix	June–August	Colony development	12333 ± 670*	0.09 ± 0.02
Narthecium ossifragum	July–August	Colony development	87500 ± 24588	0.26 ± 0.07
Hypericum perforatum	July–September	Colony development	684467 ± 293457	1.16 ± 0.5
Filipendula ulmaria	July–September	Mating phase	143713 ± 31605	0.8 ± 0.18
Calluna vulgaris	July–September	Mating phase	15126 ± 1822*	0.14 ± 0.07

Plant species are ranked in the order of the beginning of the flowering period. Bumblebee colony phase is the period during which pollen grains of the plant species were present in bumblebee loads.
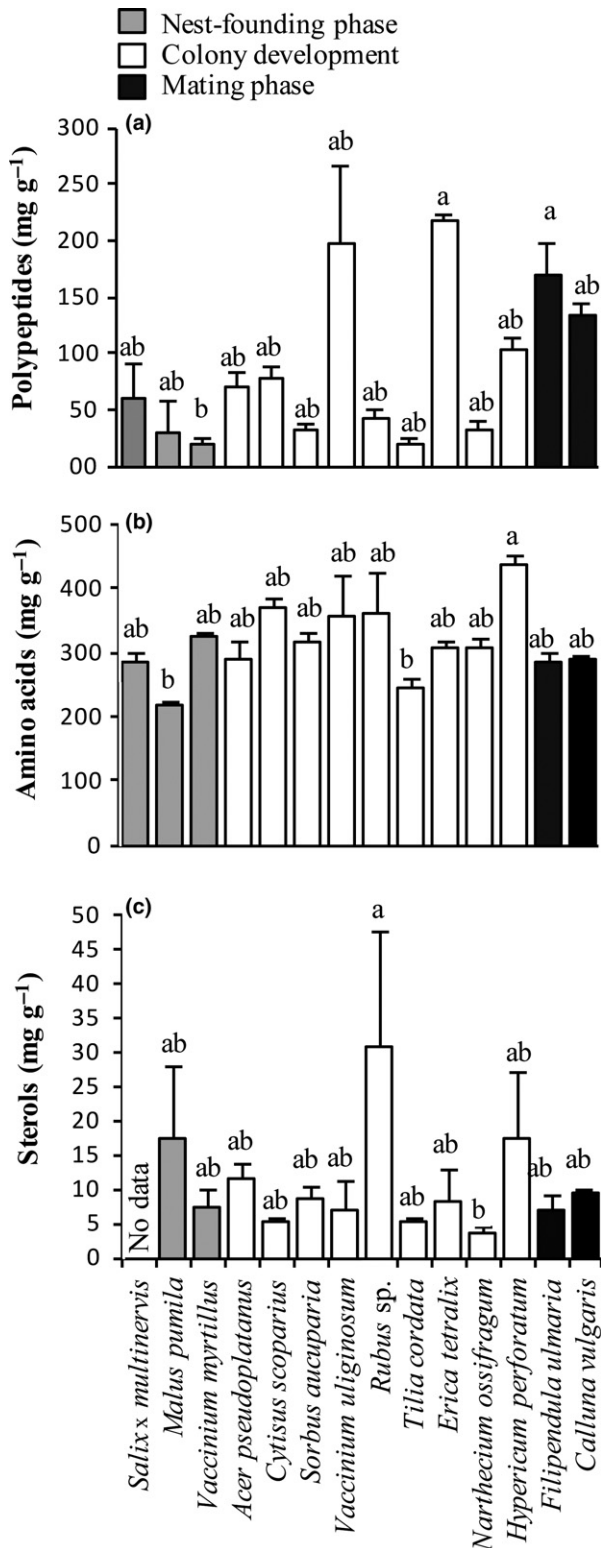*From Jacquemart (2003).

Pollen and nectar characteristics

Pollen quantity and composition. The most abundant plant species found in bumblebees pollen loads throughout the colony lifetime were *Acer pseudoplatanus* L., *Calluna vulgaris* (L.) Hull, *Cytisus scoparius* L., *Erica tetralix* L., *Filipendula ulmaria* L., *Hypericum perforatum* L., *Malus pumila* Mill., *Narthecium ossifragum* L., *Rubus* spp., *Salix* x *multinervis* Döll, *Sorbus aucuparia* L., *Tilia cordata* Mill., *Vaccinium myrtillus* L. and *Vaccinium uliginosum* L. Pollen volume per flower (Table 2) varied among species, from 0.09 ± 0. 02 mm³ (*Erica tetralix*) to 2.26 ± 0.41 mm³ (*Salix* x *multinervis*).

For pollen composition, polypeptide contents differed significantly among species (Kruskal–Wallis; χ_{13} = 39.7; $P < 0.001$; Fig. 4a; Appendix S1). For example, *V. myrtillus* pollen had significantly lower polypeptide contents (18.9 ± 7.0 mg/g) compared to *E. tetralix* (216.6 ± 6.9 mg/g; P = 0.02) and *F. ulmaria* pollen (168.7 ± 27.6 mg/g; P = 0.05). Total amino acid content also significantly differed among plant species (Kruskal–Wallis; χ_{13} = 33.0; P = 0.002; Fig. 4b). For example, *H. perforatum* pollen had higher amino acid content (434.9 ± 16.3 mg/g) than *M. pumila* (215.5 ± 14.5 mg/g; P = 0.001) and *T. cordata* (243.1 ± 13.2 mg/g; P = 0.02). Likewise, the essential amino acid composition of pollen differed significantly among plant species (PerMANOVA; $F_{13,28}$ = 12.5; P = 0.001; Appendix S2). This difference was visually assessed by PCA (Fig. 5), where plant species clustered into three groups: (i) *H. perforatum* had a high proportion of threonine and methionine and a low proportion of isoleucine and leucine; (ii) *F. ulmaria*, *Rubus* spp. and *V. uliginosum*, had a high proportion of lysine and a low proportion of phenylalanine; (iii) the last cluster included species with essential amino acid composition close to the ideal balance established by de Groot (1953).

Like protein content, sterol content (Kruskal–Wallis; χ_{12} = 25.4; P = 0.01; Fig. 4c) and composition (PerMANOVA; $F_{13,29}$ = 4.4; P = 0.001; Appendix S3) of pollen significantly differed among plant species. As illustrated by PCA (Fig. 6), plant species clustered into three groups. The first axis separated *Salix* x *multinervis* (i) from the other species and the second axis separated plant species in two groups, one (ii) composed of *A. pseudoplatanus*, *C. scoparius*, *C. vulgaris*, *E. tetralix*, *H. perforatum*, *N. ossifragum*, *T. cordata* and *V. myrtillus*, had high proportion of β-sitosterol (30–65%) and/or δ 5-avenasterol (17 to 47%). In this group, pollen of some species also had a high percentage of δ7-avenasterol (*E. tetralix* 27%, *N. ossifragum* 14%, *V. myrtillus* 12% and *C. vulgaris* 9%). The other group (iii) included the remaining species and had pollen with a high proportion of 24-methylene-cholesterol and campesterol.

Nectar quantity and composition. The nectar of the different ericaceous species had similar sugar concentrations (Kruskal–Wallis; χ_4 = 6.9578; d.f. = 4; P = 0.14; Table 3), but differed in quantity per flower (Kruskal–Wallis; χ_4 = 76.0; $P < 0.001$). *Vaccinium myrtillus* produced more nectar per flower (0.77 ± 1.38 μl) than *V. vitis-idaea* (0.12 ± 0.26 μl), *E. tetralix* (0.20 ± 0.29 μl) and *C. vulgaris* (0.01 ± 0.01 μl); indeed, *C. vulgaris* produced significantly less nectar than any other target ericaceous species. *Vaccinium myrtillus* nectar predominantly contained sucrose (87.7 ± 5.2%), whereas *V. uliginosum*, *V. vitis-idaea* and *C. vulgaris* nectar predominantly contained hexoses (glucose + fructose). *Erica tetralix* had nectar with high proportions of both fructose and sucrose.

Fig. 4. Polypeptide (a), amino acid (b) and sterol (c) contents ($n = 3$, mean ± SD; mg/g) of pollen of different plant species found in bumblebee loads. Different letters indicate significant differences between concentrations (Nemenyi *post hoc* tests, $P < 0.05$). Colours correspond to the bumblebee colony phases during which pollen grains of these plant species were present in bumblebee loads. Grey: nest-founding phase; white: colony development; black: mating phase. Plant species are ranked in the order of their flowering periods.

pollen in bumblebee loads and the composition of pollen (polypeptide, amino acid and sterol contents) or quantity of pollen (pollen volume per flower; $P > 0.05$). Fidelity to target plant species (proportion of visited target plant species and number of taxa in pollen loads) and proportion of bumblebees collecting pollen were not linked to nutrient concentrations. Bumblebee loads had similar proportion of pollen of *V. myrtillus* (0.56 ± 0.45) and *E. tetralix* (0.65 ± 0.40), despite the large difference of polypeptide concentration in their pollen (18.9 ± 7.0 vs. 216.6 ± 6.9 mg/g respectively).

In the same way, the collection of nectar was not linked to the quantity of nectar in the flower and to sugar concentration. *Calluna vulgaris*, a species visited mainly for nectar, produced the lowest quantity of nectar and this nectar had intermediate sugar concentration.

Discussion

Do ericaceous species offer sufficient pollen resources for bumblebees throughout the entire colony lifetime?

Bumblebees have been reported as the main visitors to all the target ericaceous species except *C. vulgaris*, a more generalist plant (Ritchie, 1955, 1956; Jacquemart, 1993; Mahy *et al.*, 1998; Mayer *et al.*, 2012). In this study, bumblebees visited ericaceous species for pollen and/or nectar, but not all ericaceous species were visited for the same floral resources: the bumblebees mainly visited *V. vitis-idaea* for pollen and *C. vulgaris* for nectar. The different flowering periods could explain these differences, since the flowering period of *V. vitis-idaea* corresponds to bumblebee colony development (Prŷs-Jones & Corbet, 1987), when pollen collection is important for larval growth (Ribeiro *et al.*, 1998; Pelletier & McNeil, 2003). By contrast, the flowering period of *C. vulgaris* corresponds to the mating phase and the end of the colony lifetime, when males and queens forage for their own needs (Prŷs-Jones & Corbet, 1987). Furthermore, bumblebee fidelity for pollen from the studied heathland species was higher in the early spring on *S.* x *multinervis* (Moquet *et al.*, 2015) and in the late summer on *C. vulgaris*. These results suggested that the food web of bumblebees in heathlands is highly limited (Mayer *et al.*, 2012) during these two periods.

The foraging behaviour differed according to bumblebee species. Ericaceous species were the main sources of
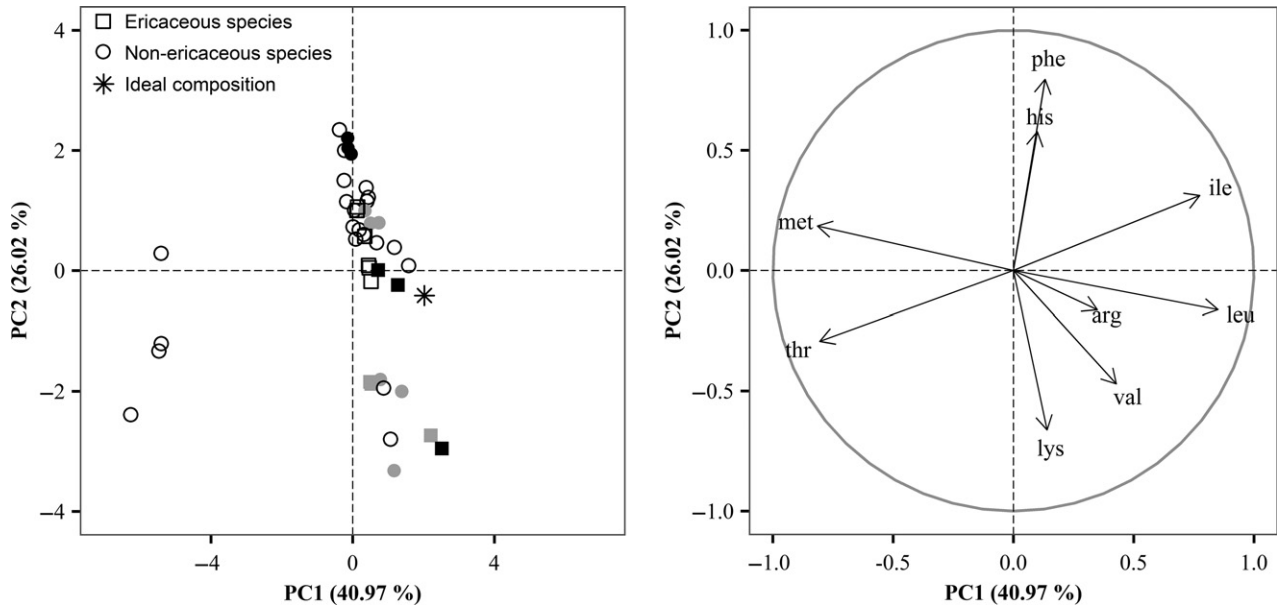
Links between nutritional content and bumblebee diet

Despite differences in pollen quantity and quality, we did not detect any correlation between the proportions of

Fig. 5. Principal component analysis calculated on percentage of total of essential amino acids in pollen from the target plant species. The relative contributions of each principal component for the total variance of the data set are shown in brackets. Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Val = valine. Bumblebee colony phases in which these species were present in the bumblebee diet are indicated as follows: Solid grey dots: nest founding phase; empty dots: colony development; solid black dots: mating phase.

Fig. 6. Principal component analysis calculated on percentage of total sterol content in pollen. The relative contributions of each principal component for the total variance of the data set are shown in brackets. Bumblebee colony phases in which these species were present in the bumblebee diet are indicated. Solid grey dots: nest-founding phase; empty dots: colony development; solid black dots: mating phase. Sterol identification: 1, cholesterol; 2, desmosterol; 3, 24-methylenecholesterol and campesterol; 4, stigmasterol; 5, unknown 1 (mass TMS = 484); 6, β-sitosterol; 7, δ5-avenasterol; 8, cholestenone; 9, δ7-stigmasterol; 10, δ7-avenasterol; 11, unknown 2; 12, unknown 3.

both pollen and nectar for *B. jonellus* throughout the colony lifetime and around 40% of individuals carried pollen loads with a mean of 80% of ericaceous pollen grains.

Bombus jonellus showed higher fidelity for ericaceous species than did other bumblebee species, particularly *B. terrestris*. Moreover, the proportion of heathland plant

Table 3. Nectar production of the five target ericaceous plant species: quantity of nectar per flower (µl; mean $\pm$ SD), total sugar content (g/100 g; mean $\pm$ SD) and glucose, fructose and sucrose percentage.

Plant species	Quantity per flower (µl)	Sugar content (g/100 g)	Glucose (%)	Fructose (%)	Sucrose (%)
V. myrtillus	0.8 ± 1.4	17.9 ± 6.1	4.7 ± 2.9	7.6 ± 2.5	87.7 ± 5.2
V. uliginosum	0.3 ± 0.4	27.4 ± 11.2	40.2 ± 2.3	59.0 ± 1.7	0.8 ± 0.6
V. vitis-idaea	0.1 ± 0.3	21.1 ± 12.8	44.9 ± 1.6	52.6 ± 1.3	2.6 ± 0.9
E. tetralix	0.2 ± 0.3	6.0 ± 3.8	7.9 ± 1.1	44.9 ± 3.4	47.2 ± 2.3
C. vulgaris	0.0 ± 0.0	16.6 ± 6.7	43.4 ± 1.5	53.3 ± 0.7	3.3 ± 0.8

All sites were pooled. $n = 3$ replicates for nectar ratio and composition. $n > 60$ floral units for nectar quantities. Plant species are ranked in the order of the flowering period.

species was higher in the diet of *B. jonellus* than in the diet of other bumblebee species. These results can be explained by *B. jonellus* having a more restricted preference for foraging on ericaceous species (Goulson *et al.*, 2005; Kleijn & Raemakers, 2008) or having smaller foraging distances compared with *B. terrestris* (Martin *et al.*, 2005; Westphal *et al.*, 2006).

By contrast, other bumblebee species showed lower fidelity to ericaceous species. For example, *B. pascuorum* visited ericaceous species mainly for nectar, as a low proportion of observed individuals had pollen loads. *Bombus hypnorum*, which usually prefer foraging from tree species (Crowther *et al.*, 2014), was observed in large amounts on *V. vitis-idaea*, where individuals mainly collected pollen. Like *B. pascuorum*, *B. hypnorum* was dependent on the surrounding biotopes for one flower resource or for both pollen and nectar. Moreover, plant species present in the pollen loads indicated that bumblebees move between different elements in the landscape mosaic, including heathlands, peatlands, meadows and their margins. This result highlights the importance of heterogeneity for bumblebee foraging in a mosaic landscape (Westphal *et al.*, 2006; Goulson *et al.*, 2010; Somme *et al.*, 2014; Senapathi *et al.*, 2015).

Is the nutritional composition of pollen valuable throughout the colony lifetime?

The pollen of plant species differed significantly in polypeptide, amino acid and sterol contents. For example, *E. tetralix* and *F. ulmaria* pollen were characterised by high polypeptide but pollen of *V. myrtillus*, which flowered during bumblebee nest foundation, had a very low polypeptide contents. In contrast to the previous study from Roulston *et al.* (2000), our study showed a significant difference in polypeptide contents among species of the same plant family (Ericaceae) and high variability among species of the same genus (*Vaccinium*). Despite the differences in polypeptide concentrations, the amino acid and essential amino acid concentrations of the different ericaceous pollens were high and remained similar throughout colony lifetime. We, however, observed differences in amino acid contents among other species found in pollen loads. For example, *H. perforatum* had a higher amino acid content than *M. pumila* and *T. cordata*. The

majority of pollen had comparable essential amino acid compositions, although several species present in significant proportion in pollen loads had pollen with lower concentrations of some essential amino acids, for instance *H. perforatum*, *F. ulmaria* and *Rubus* spp.

The sterol content of ericaceous pollen was similar throughout colony lifetime. Sterol contents of the diet can affect insect growth (Pilorget *et al.*, 2010; Vanderplanck *et al.*, 2014b), but the quantity needed for bumblebee growth remains unknown. Our studied plant species can be separated into two groups according to sterol composition: the first group included the target ericaceous species, in which β-sitosterol and/or δ5-avenasterol were dominant and the second group included species in which 24-methylenecholesterol was dominant. High contents of 24-methylenecholesterol, β-sitosterol or δ5-avenasterol can promote the development of bigger larvae in *B. terrestris* (Vanderplanck *et al.*, 2014b). The essential sterol 24-methylcholesterol influences moulting and the development of ovaries (Svoboda *et al.*, 1978, 1980; Human *et al.*, 2007), and β-sitosterol and δ5-avenasterol have a phagostimulant effect on bumblebee species (Rasmont *et al.*, 2005). Pollen of *E. tetralix*, *N. ossifragum*, *V. myrtillus* and *C. vulgaris* contained a significant amount of δ7-avenasterol (9–27%). δ7-phytosterol might act as a chemical protectant for pollen, as it has been reported to be detrimental to herbivorous insects lacking the enzymes necessary to completely convert δ7-sterols to δ5-sterols (Janson *et al.*, 2009; Sedivy *et al.*, 2011).

Therefore, the target ericaceous species studied here provide valuable resources throughout the colony lifetime. In fact, all studied species had pollen with total amino acid contents higher than 20%, a value considered to promote the development of bee larvae (Génissel *et al.*, 2002; Tasei & Aupinel, 2008; Vanderplanck *et al.*, 2014a). Nevertheless, *V. myrtillus* pollen had relatively low polypeptide contents, and some species having pollen rich in δ7-sterol, which may be detrimental for development of bee larvae.

Is the nutritional composition of nectar from ericaceous species valuable throughout the colony lifetime?

Bees do not often collect pollen and nectar simultaneously, but typically visit different plant species for each

resource (Brian, 1957). In our study, some ericaceous species like *C. vulgaris* were mainly visited for nectar.

The sugar content of nectar did not differ significantly among species and was lower than 40%, the concentration usually preferred by bumblebees (Harder, 1986; Cnaani *et al.*, 2006). Consequently, total sugar content cannot explain differences in preference among the bumblebee species studied. Here, we found that the quantity of nectar differed among plant species, with *V. myrtillus* flowers providing significantly more nectar than other species. It is possible that, because *V. myrtillus* had low pollen quality in comparison to other species, high nectar quantity is the main attractive reward. In contrast, *C. vulgaris* produced very little nectar per flower, but due to its high flower density (up to 7900 flowers per individual, Beijerinck, 1940; Gimingham, 1960), the total quantity remained attractive.

The sugar composition of nectar also differed among ericaceous species. Nectar of *V. myrtillus* was sucrose dominant, whereas nectars of *V. uliginosum*, *V. vitis-idaea* and *C. vulgaris* were hexose dominant. Different sugar compositions in nectar within the same family or genus have been described previously (Barnes *et al.*, 1995; Krömer *et al.*, 2008). The nectar of *E. tetralix* is quite unusual, even within the *Erica* genus, offering both fructose and sucrose in large amounts (Baker & Baker, 1990; Barnes *et al.*, 1995; Krömer *et al.*, 2008). In contrast to other studies (Percival, 1961; Baker & Baker, 1983, 1990; Krömer *et al.*, 2008), we did not find any relationship between the nectar composition and floral visitors (i.e. bumblebees for all target plants) or between the nectar composition and floral morphology (e.g. *V. vitis-idaea* and *C. vulgaris* have open flowers).

What governs bumblebee fidelity and choice of pollen resources?

We observed no correlation between the abundance of pollen in bumblebee loads and the concentrations of polypeptides, amino acids and sterols or the pollen volume. Similarly, neither the proportion of bumblebees collecting pollen of ericaceous species nor the fidelity was affected by polypeptide, amino acid or sterol concentrations. For example, the bumblebees showed similar fidelity for *V. myrtillus* and *E. tetralix*, despite the large difference in polypeptide concentration in their pollen. This observation disagrees with results of other studies showing the importance of pollen quality in bumblebee choices (Robertson *et al.*, 1999; Hanley *et al.*, 2008; Kitaoka & Nieh, 2008; Moquet *et al.*, 2015). It is now clear that bumblebees can distinguish between pollen with low or high protein content (Ruedenauer *et al.*, 2015). Bumblebees can use this capacity to choose plants with high pollen quality when they have a binary choice (Robertson *et al.*, 1999; Kitaoka & Nieh, 2008; Moquet *et al.*, 2015; Quinet *et al.*, 2016). In field observations, when many flowering species are

available, bumblebee choices were less clear (Rasheed & Harder, 1997).

The lack of correlation between pollen quality and the proportion of specific pollen in the bumblebee diet can be explained by the need to mix pollen of different origins. Pollen mixing increases the quality of the overall diet by improving the nutrient balance (Bernays *et al.*, 1994; Eckhardt *et al.*, 2013). In our study, some bumblebee species supplemented pollen poor in 24-methylenecholesterol but rich in δ7-sterols (from *V. myrtillus*, *E. tetralix* and *C. vulgaris*) with pollen rich in 24-methylenecholesterol and campesterol (from *F. ulmaria*, *Malus pumila* or *Rubus* spp.). In the same way, the bumblebees combined different pollen amino acid profiles. Moreover, Rasheed and Harder (1997) showed that pollen selection is not only exclusively dependent on pollen composition, but also depends on other factors such as plant density (Kunin, 1997) or spatial distribution (Jha *et al.*, 2013).

Conclusion

Our results demonstrate that heathlands provide valuable habitats for bumblebees due to the succession of flowering periods and the presence of suitable nutrients in pollen and nectar of ericaceous species. The high fidelity of bumblebees, particularly of *B. jonellus*, for ericaceous species showed that these plant species can constitute a substantial part of the bumblebee pollen diet in heathlands. Nevertheless, despite European legislation, the quality and quantity of heathland continues to decrease in Europe (Romão *et al.*, 2015). Estimation of the minimal heathland area needed to maintain bumblebee populations, by determining the quantity of ericaceous pollen required for colony development, might provide an important topic for future research.

Our results also indicate the importance of conservation of the landscape mosaic at the bumblebee foraging scale. All bumblebee species harvest pollen on species growing outside heathlands, including *Rubus*, *Sambucus* and *Trifolium*. Nevertheless, we detected no relationship between the abundance of a particular plant species in the bumblebee diet and the nutritional content of its pollen. These results show that other factors might determine foraging behaviour, although pinpointing these factors will require additional research. Quantification of floral resources at the landscape level is needed to establish bumblebee nutritional requirements and choices and to understand their foraging behaviour.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12201:

Appendix S1. Polypeptide, amino acid, essential amino acid and sterol contents (mean ± SD; mg/g) of pollen of different plant species found in bumblebee loads in the studied heathland sites. Plant species are ranked in the order of flowering.

Appendix S2. Essential amino acid composition of pollen of 14 plant species found in bumblebee loads in the studied heathland sites, expressed as percentage of total of essential amino acids (mean ± SD). Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Val = valine. Plant species are ranked in the order of flowering.

Appendix S3. Sterol composition of pollen of 14 plant species found in bumblebee loads in the studied heathland sites, expressed as percentage of total sterol content. 24-methyl + camp = 24-methylenecholesterol and campesterol. Plant species are ranked in the order of flowering.

References

Aerts, R. & Heil, G.W. (1993) *Heathlands: patterns and processes in a changing environment*. Kluwer Academic Publishers, Dordrecht.

Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010) Diet effects on honeybee immunocompetence. *Biology Letters*, **6**, 562–565.

Alaux, C., Jaisson, P. & Hefetz, A. (2005) Reproductive decision-making in semelparous colonies of the bumblebee *Bombus terrestris*. *Behavioral Ecology and Sociobiology*, **59**, 270–277.

Aupinel, P., Genissel, A., Tasei, J.-N., Poncet, J. & Gomond, S. (2001) Collection of spring pollens by *Bombus terrestris* queens. Assessment of attractiveness and nutritive value of pollen diets. *Acta Horticulturae*, **561**, 101–105.

Baker, H.G. & Baker, I. (1983) Floral nectar sugar constituents in relation to pollinator type. *Handbook of Experimental Pollination Biology*, pp. 117–141. (ed. by C.E. Jones and R.J. Little), Van Nostrand Reinhold, New York.

Baker, H.G. & Baker, I. (1990) The predictive value of nectar chemistry to the recognition of pollinator types. *Israel Journal of Botany*, **39**, 157–166.

Baldock, K.C.R., Goddard, M.A., Hicks, D.M., Kunin, W.E., Mitschunas, N., Osgathorpe, L.M., Potts, S.G., Robertson, K.M., Scott, A.V., Stone, G.N., Vaughan, I.P. & Memmott, J. (2015) Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20142849.

Barnes, K., Nicolson, S.W. & Van Wyk, B.-E. (1995) Nectar sugar composition in *Erica. Biochemical Systematics and Ecology*, **23**, 419–423.

Behmer, S.T. & Nes, W.D. (2003) Insect sterol nutrition and physiology: a global overview. *Advances in Insect Physiology*, **31**, 1–72.

Beijerinck, W. (1940) *Calluna*. A monograph on the Scotch heather. *Verhandelingen Akademie van Wetenscappen*, **38**, 1–180.

Bennett, A.B. & Isaacs, R. (2014) Landscape composition influences pollinators and pollination services in perennial biofuel plantings. *Agriculture, Ecosystems and Environment*, **193**, 1–8.

Benton, T. (2006) *Bumblebees: the natural history and identification of the species found in Britain*. Collins, London.

Bernays, E.A., Bright, K.L., Gonzalez, N. & Angel, J. (1994) Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology*, **75**, 1997–2006.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R. & Thomas, C.D. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351–354.

Bloch, G., Hefetz, A. & Hartfelder, K. (2000) Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*). *Journal of Insect Physiology*, **46**, 1033–1040.

Brian, A.D. (1957) Differences in the flowers visited by four species of bumble-bees and their causes. *Journal of Animal Ecology*, **26**, 71–98.

Buchmann, S.L. (1983) Buzz pollination in Angiosperms. *Handbook of experimental pollination biology*, pp. 73–113. (ed. by C.E. Jones and R.J. Little), Van Nostrand Reinhold, New York.

Buchmann, S.L. & O'rourke, M.K. (1991) Importance of pollen grain volumes for calculating bee diets. *Grana*, **30**, 591–595.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., Goffe, L. & Darvill, B. (2011) Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, **108**, 662–667.

Carvalheiro, L.G., Kunin, W.E., Keil, P., Aguirre-Gutiérrez, J., Ellis, W.N., Fox, R., Groom, Q., Hennekens, S., Van Landuyt, W., Maes, D., Van de Meutter, F., Michez, D., Rasmont, P., Ode, B., Potts, S.G., Reemer, M., Roberts, S.P.M., Schaminée, J., WallisDeVries, M.F. & Biesmeijer, J.C. (2013) Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. *Ecology Letters*, **16**, 870–878.

Cnaani, J., Thomson, J.D. & Papaj, D.R. (2006) Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. *Ethology*, **112**, 278–285.

Cook, S.M., Awmack, C.S., Murray, D.A. & Williams, I.H. (2003) Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecological Entomology*, **28**, 622–627.

Corbet, S.A., Williams, I.H. & Osborne, J.L. (1991) Bees and the pollination of crops and wild flowers in the European Community. *Bee World*, **72**, 47–59.

Crowther, L.P., Hein, P.-L. & Bourke, A.F.G. (2014) Habitat and forage associations of a naturally colonising insect pollinator, the tree bumblebee *Bombus hypnorum*. *PLoS ONE*, **9**, e107568.

Duchateau, M.J. & Velthuis, H.H.W. (1988) Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour*, **107**, 186–207.

Eckhardt, M., Haider, M., Dorn, S. & Müller, A. (2013) Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? *The Journal of Animal Ecology*, **83**, 588–597.

Erdtman, G. (1960) The acetolysis method. *A revised description. Svensk Botanisk Tidskrift*, **54**, 561–564.

European Commission. (1979) Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds. *Official Journal*, **103**, 103–135.

European Commission. (1992) Council directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal*, **206**, 7–49.

Fahrig, L. (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 487–515.

Forup, M.L., Henson, K.S., Craze, P.G. & Memmott, J. (2007) The restoration of ecological interactions: plant–pollinator networks on ancient and restored heathlands. *Journal of Applied Ecology*, **45**, 742–752.

Génissel, A., Aupinel, P., Bressac, C., Tasei, J.-N. & Chevrier, C. (2002) Influence of pollen origin on performance of *Bombus terrestris* micro-colonies. *Entomologia Experimentalis et Applicata*, **104**, 329–336.

Gilbert, F.S. (1981) Foraging ecology of hoverflies: morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. *Ecological Entomology*, **6**, 245–262.

Gimingham, C.H. (1960) *Calluna* Salisb. *Journal of Ecology*, **48**, 455–483.

Goodwin, S.G. (1995) Seasonal phenology and abundance of early-, mid- and long-season bumble bees in southern England, 1985–1989. *Journal of Apicultural Research,* **34**, 79–87. Goulson, D. (2000) Are insects flower constant because they use search images to find flowers? *Oikos*, **88**, 547–552.

Goulson, D. & Darvill, B. (2004) Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? *Apidologie*, **35**, 55–63.

Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. & Knight, M.E. (2005) Causes of rarity in bumblebees. *Biological Conservation*, **122**, 1–8.

Goulson, D., Lepais, O., O'Connor, S., Osborne, J.L., Sanderson, R.A., Cussans, J., Goffe, L. & Darvill, B. (2010) Effects of land use at a landscape scale on bumblebee nest density and survival. *Journal of Applied Ecology*, **47**, 1207–1215.

Goulson, D., Nicholls, E., Rotheray, E. & Botias, C. (2015) Qualifying pollinator decline evidence—Response. *Science*, **348**, 982–982.

Grixti, J.C., Wong, L.T., Cameron, S.A. & Favret, C. (2009) Decline of bumble bees (*Bombus*) in the North American Midwest. *Biological Conservation*, **142**, 75–84.

de Groot, A.P. (1953) Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). *Physiologia Comparata et Oecologia*, **3**, 1–90.

Hanley, M.E., Franco, M., Pichon, S., Darvill, B. & Goulson, D. (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Functional Ecology*, **22**, 592–598.

Harder, L.D. (1986) Effects of nectar concentration and flower depth on flower handling efficiency of bumble bees. *Oecologia*, **69**, 309–315.

Harris, L.F. & Johnson, S.D. (2004) The consequences of habitat fragmentation for plant-pollinator mutualisms. *International Journal of Tropical Insect Science*, **24**, 29–43.

Hendrickx, F., Maelfait, J.-P., Van Wingerden, W., Schweiger, O., Speelmans, M., Aviron, S., Augenstein, I., Billeter, R., Bailey, D., Bukacek, R., Burel, F., Diekötter, T., Dirksen, J., Herzog, F., Liira, J., Roubalova, M., Vandomme, V. & Bugter, R. (2007) How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. *Journal of Applied Ecology*, **44**, 340–351.

Hermann, P.M. & Palser, B.F. (2000) Stamen development in the Ericaceae. I. Anther wall, microsporogenesis, inversion, and appendages. *American Journal of Botany*, **87**, 934–957.

Human, H., Nicolson, S.W., Strauss, K., Pirk, C.W.W. & Dietemann, V. (2007) Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). *Journal of Insect Physiology*, **53**, 649–655.

Jacquemart, A.-L. (1993) Floral visitors of *Vaccinium species* in the high Ardenne, Belgium. *Flora*, **188**, 263–273.

Jacquemart, A.-L. (2003) Floral traits of Belgian Ericaceae species: are they good indicators to assess the breeding systems? *Belgian Journal of Botany*, **136**, 154–164.

Janson, E.M., Grebenok, R.J., Behmer, S.T. & Abbot, P. (2009) Same host-plant, different sterols: variation in sterol metabolism in an insect herbivore community. *Journal of Chemical Ecology*, **35**, 1309–1319.

Jha, S., Stefanovich, L. & Kremen, C. (2013) Bumble bee pollen use and preference across spatial scales in human-altered landscapes. *Ecological Entomology*, **38**, 570–579.

Kitaoka, T.K. & Nieh, J.C. (2008) Bumble bee pollen foraging regulation: role of pollen quality, storage levels, and odor. *Behavioral Ecology and Sociobiology*, **63**, 501–510.

Kleijn, D. & Raemakers, I. (2008) A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology*, **89**, 1811–1823.

Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Krol, W., Solarz, W. & Plonka, P. (2007) The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. *Oryx*, **41**, 79–88.

Krömer, T., Kessler, M., Lohaus, G. & Schmidt-Lebuhn, A.N. (2008) Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. *Plant Biology*, **10**, 502–511.

Kunin, W.E. (1997) Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica* Kaber. *Journal of Ecology*, **85**, 225–234.

Lambinon, J. & Verloove, F. (2012) *Nouvelle flore de Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des régions voisines*, Sixième edn. Jardin botanique national de Belgique, Meise.

Mahy, G., De Sloover, J.R. & Jacquemart, A.-L. (1998) The generalist pollination system and reproductive success of *Calluna vulgaris* in the Upper Ardenne. *Canadian Journal of Botany*, **76**, 1843–1851.

Martin, A.P., Bishop, S., Osborne, J.L., Hale, R.J., Sanderson, R.A. & Goulson, D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Molecular Ecology*, **14**, 1811–1820.

Mayer, C., Michez, D., Chyzy, A., Brédat, E. & Jacquemart, A.-L. (2012) The abundance and pollen foraging behaviour of bumble bees in relation to population size of whortleberry (*Vaccinium uliginosum*). *PLoS ONE*, **7**, e50353.

Moerman, R., Vanderplanck, M., Roger, N., Declèves, S., Wathelet, B., Rasmont, P., Fournier, D. & Michez, D. (2015) Growth rate of bumblebee larvae is related to pollen amino acids. *Journal of Economic Entomology*, **109**, 25–30.

Moisan-Deserres, J., Girard, M., Chagnon, M. & Fournier, V. (2014) Pollen loads and specificity of native pollinators of lowbush blueberry. *Journal of Economic Entomology*, **107**, 1156–1162.

Moquet, L., Mayer, C., Michez, D., Wathelet, B. & Jacquemart, A.-L. (2015) Early spring floral foraging resources for pollinators in wet heathlands in Belgium. *Journal of Insect Conservation*, **19**, 837–848.

Nicolson, S.W. (2011) Bee food : the chemistry and nutritional value of nectar, pollen and mixtures of the two : review article. *African Zoology*, **46**, 197–204.

Nieto, A., Roberts, S.P.M., Kemps, J., Rasmont, P., Kuhlmann, M., García Criado, M., Biesmeijer, J.C., Bogusch, P., Dathe, H.H., De la Rua, P., De Meulemeester, T., Dehon, M., Dewulf, A., Ortiz-Sánchez, F.J., Lhomme, P., Pauly, A., Potts, S.G., Praz, C., Quaranta, M., Radchenko, V.G., Scheuchl, E., Smit, J., Straka, J., Terzo, M., Bogdan, J. & Window, J. & Michez, D. (2015) *European Red List of Bees*. Publication Office of the European Union, Luxembourg.

Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos*, **120**, 321–326.

Osborne, J.L. & Williams, I.H. (1996) Bumble bees as pollinators of crops and wild flowers. *Bumble bees for pleasure and profit*, pp. 24–32. (ed. by A. Matheson), Cardiff, Ibra.

Pelletier, L. & McNeil, J.N. (2003) The effect of food supplementation on reproductive success in bumblebee field colonies. *Oikos*, **103**, 688–694.

Percival, M.S. (1961) Types of nectar in Angiosperms. *New Phytologist*, **60**, 235–281.

Pilorget, L., Buckner, J. & Lundgren, J.G. (2010) Sterol limitation in a pollen-fed omnivorous lady beetle (Coleoptera: Coccinellidae). *Journal of Insect Physiology*, **56**, 81–87.

Prŷs-Jones, O.E. & Corbet, S.A. (1987) *Bumblebees*. The Richmond Publishing Company Ltd, Slough.

Quinet, M., Warzée, M., Vanderplanck, M., Michez, D., Lognay, G. & Jacquemart, A.-L. (2016) Do floral resources influence pollination rates and subsequent fruit set in pear (*Pyrus communis* L.) and apple (*Malus* x *domestica* Borkh) cultivars? *European Journal of Agronomy*, **77**, 59–69.

R Development Core Team. (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rasheed, S. & Harder, L. (1997) Economic motivation for plant species preferences of pollen-collecting bumble bees. *Ecological Entomology*, **22**, 209–219.

Rasmont, P., Leclercq, J., Jacob-Remacle, A., Pauly, A. & Gaspar, C. (1993) The faunistic drift of Apoidea in Belgium. *Bees for pollination* (ed. by E. Bruneau), pp. 65–87. Commission of the EC, Brussel, Belgium.

Rasmont, P., Regali, A., Ings, T.C., Lognay, G., Baudart, E., Marlier, M., Delcarte, E., Viville, P., Marot, C. & Falmagne, P. (2005) Analysis of pollen and nectar of *Arbutus unedo* as a food source for *Bombus terrestris* (Hymenoptera: Apidae). *Journal of Economic Entomology*, **98**, 656–663.

Rasmont, P. & Terzo, M. (2010) *Catalogue et clé des sous-genres et espèces du genre Bombus de Belgique et du nord de la France (Hymenoptera, Apoidea)*. Université de Mons, Laboratoire de Zoologie.

Rathcke, B.J. & Jules, E.S. (1993) Habitat fragmentation and plant-pollinator interactions. *Current Science*, **65**, 273–277.

Rebane, M., Wynde, R., Diemont, W.H., Jensen, F.P., Pahlsson, L. & Webb, N.R. (1997) Lowland Atlantic heathland. *Habitats for birds in Europe: a conservation strategy for the wider environment*, pp. 187–202. (ed. by G.M. Tucker and M.I. Evans), Cambridge, UK, Birdlife International.

Reille, M. (1992) *Pollen et spores d'Europe et d'Afrique du Nord*. Laboratoire de Botanique Historique et Palynologie, Marseille.

Ribeiro, M.F., Velthuis, H.H.W., Duchateau, M.J. & van der Tweel, I. (1998) Feeding frequency and caste differentiation in *Bombus terrestris* larvae. *Insectes Sociaux*, **46**, 306–314.

Ritchie, J.C. (1955) *Vaccinium vitis-idaea* L. *Journal of Ecology*, **43**, 701–708.

Ritchie, J.C. (1956) *Vaccinium myrtillus* L. *Journal of Ecology*, **44**, 291–299.

Robertson, A.W., Mountjoy, C., Faulkner, B.E., Roberts, M.V. & Macnair, M.R. (1999) Bumble bee selection of *Mimulus guttatus* flowers: the effects of pollen quality and reward depletion. *Ecology*, **80**, 2594–2606.

Romão, C., Graf, A., Naumann, S., McKenna, D., Evans, D., Richard, D., Bailly, J. & Gaudillat-Sipkova, Z. (2015) *State of nature in the EU — European Environment Agency*. European Environment Agency, Denmark.

Roulston, T.H. & Cane, J.H. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, **222**, 187–209.

Roulston, T.H., Cane, J.H. & Buchmann, S.L. (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, **70**, 617–643.

Ruedenauer, F.A., Spaethe, J. & Leonhardt, S.D. (2015) How to know which food is good for you: bumblebees use taste to discriminate between different concentrations of food differing in nutrient content. *The Journal of Experimental Biology*, **218**, 2233–2240.

Sedivy, C., Müller, A. & Dorn, S. (2011) Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. *Functional Ecology*, **25**, 718–725.

Senapathi, D., Carvalheiro, L.G., Biesmeijer, J.C., Dodson, C.-A., Evans, R.L., McKerchar, M., Morton, R.D., Moss, E.D., Roberts, S.P.M., Kunin, W.E. & Potts, S.G. (2015) The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. *Proceedings of the Royal Society of London, B*, **282**, 20150294.

Smeets, P. & Duchateau, M.J. (2003) Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. *Apidologie*, **34**, 333–337.

Somme, L., Vanderplanck, M., Michez, D., Lombaerde, I., Moerman, R., Wathelet, B., Wattiez, R., Lognay, G. & Jacquemart, A.-L. (2014) Pollen and nectar quality drive the major and minor floral choices of bumble bees. *Apidologie*, **46**, 1–15.

Svoboda, J.A., Thompson, M.J., Herbert, E.W. Jr & Shimanuki, H. (1980) Sterol utilization in honey bees fed a synthetic diet: Analysis of prepupal sterols. *Journal of Insect Physiology*, **26**, 291–294.

Svoboda, J.A., Thompson, M.J., Robbins, W.E. & Kaplanis, J.N. (1978) Insect steroid metabolism. *Lipids*, **13**, 742–753.

Tasei, J.-N. & Aupinel, P. (2008) Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie*, **39**, 397–409.

Terzo, M. & Rasmont, P. (2007) *MALVAS, suivi, étude et vulgarisation sur l'interaction entre les MAE et les abeilles sauvages*. Direction Générale de l'Agriculture, Université de Mons, Région Wallonne.

Vanderplanck, M., Leroy, B., Wathelet, B., Wattiez, R. & Michez, D. (2014a) Standardized protocol to evaluate pollen polypeptides as bee food source. *Apidologie*, **45**, 1–13.

Vanderplanck, M., Michez, D., Vancraenenbroeck, S. & Lognay, G. (2011) Micro-quantitative method for analysis of sterol levels in honeybees and their pollen loads. *Analytical Letters*, **44**, 1807–1820.

Vanderplanck, M., Moerman, R., Rasmont, P., Lognay, G., Wathelet, B., Wattiez, R. & Michez, D. (2014b) How does pollen chemistry impact development and feeding behaviour of polylectic bees? *PLoS ONE*, **9**, e86209.

Webb, N.R. (1998) The traditional management of European heathlands. *Journal of Applied Ecology*, **35**, 987–990.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2006) Bumblebees experience landscapes at different spatial scales: possible implications for coexistence. *Oecologia*, **149**, 289–300.