

Global patterns in bumble bee pollen collection show phylogenetic conservation of diet

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Abstract

1. Bumble bees (*Bombus*) are a group of eusocial bees with a strongly generalised feeding pattern, collecting pollen from many different botanical families. Though predominantly generalists, some bumble bee species seem to have restricted dietary choices. It is unclear whether restricted diets in bumble bees are inherent or a function of local conditions due to a lack of data for many species across different regions.
2. The objective of this study was to determine whether bumble bee species displayed specific patterns of pollen collection, and whether patterns were influenced by phylogenetic relatedness or tongue length, a trait known to be associated with structuring floral visitation.
3. Bumble bee pollen collection patterns were quantified from 4,132 pollen loads taken from 58 bumble bee species, representing 24% of the pollen-collecting diversity of this genus.
4. Phylogenetic trait mapping showed a conserved pattern of dietary dissimilarity across species, but not for dietary breadth. Dietary dissimilarity was driven by collection of Fabaceae, with the most similar species collecting around 50%–60% of their diet from this botanical family. The proportion of the diet collected from Fabaceae also showed a conserved phylogenetic signal. Greater collection of Fabaceae was associated with longer tongue lengths, with shorter tongued species focusing on alternative botanical families. However, this result was largely driven by phylogenetic relatedness, not tongue length per se.
5. These results demonstrate that, though generalists, bumble bees are still subject to dietary restrictions that constrain their foraging choices. These dietary constraints have implications for their persistence should their core resources decline in abundance.

KEYWORDS

Bombus, dietary specialisation, Fabaceae, museum specimens, pollen analysis, tongue length

1 | INTRODUCTION

Herbivorous insects exhibit one of the most successful evolutionary strategies on Earth, comprising one in every three described eukaryotic species (Hardy et al., 2020). Though pronounced dietary generalists exist, the majority of insect herbivores are strongly specialised and using a limited number of host plants, typically within single botanical families (Forister et al., 2015; Jaenike, 1990). In addition to this use of specific host plants, phylogenetically conserved dietary patterns are commonly observed within clades of herbivores, with related insects feeding on related plants. This phenomenon has been most clearly studied in the Lepidoptera (Ehrlich & Raven, 1964; Janz & Nylin, 1998), but should apply equally across all groups as they are all subject to the same fundamental selective pressures that shape their dietary choices (Hardy et al., 2020; Hunter, 2016).

Against this context, bees (Hymenoptera: Anthophila) represent an ideal group in which to further investigate the phenomenon of conserved dietary specialisation. Bees are a diverse lineage that have long been the subject of scientific study because of their strong mutualism with flowering plants. As the single most important insect pollinators (Rader et al., 2016), bees drive the sexual reproduction of a large proportion of the nearly 90% of animal-pollinated flowering plant species (Ollerton et al., 2011) through pollination. This pollination occurs during the concurrent collection of the resources necessary to produce their own offspring. With very few exceptions, bees feed their developing larvae entirely on plant pollen, nectar, and in some cases, oils. Bees must therefore be classed as herbivorous insects, at least functionally if not strictly trophically (Steffan et al., 2019). Though bees are herbivores, they have not traditionally been used as models for understanding the evolution of herbivory in insects, this being much more extensively studied in the Lepidoptera (Ehrlich & Raven, 1964; Forister et al., 2015; Jaenike, 1990). Bee-plant interactions are predominantly studied in the context of pollination, which should be classified as a balanced mutual exploitation due to the potential for reduced plant fitness resulting from overharvesting of pollen (Westerkamp, 1996), and pollination syndromes that exploit bees (Dafni, 1984).

Specialised patterns of pollen collection by bees are well known and have been documented for almost a century (Robertson, 1925). It was classically suggested that bees have evolved towards pollen specialisation from generalist ancestors (Moldenke, 1979). However, the current consensus is that generalised species predominantly emerge from specialised clades which are characterised by the conservation of pollen specialisation, with occasional switches to novel hosts (Dellicour et al., 2014; Müller, 1996; Patiny et al., 2007; Sipes & Tepedino, 2005). Examples of the phylogenetic conservation of pollen collection suggest that physiological or neurological constraints exist that limit the ability of bee species and clades to break away

from their ancestral floral hosts (Sedivy et al., 2008). To date, most studies involve bee lineages that are dominated by specialists that collect pollen from a single botanical family. From first principles, it would be expected that these constraints should also have an observable effect on lineages dominated by generalised species, as suggested within a lineage of megachilid bees (Haider et al., 2014). However, this has not been investigated elsewhere, in part because of the lack of robust empirical data quantifying pollen collection patterns in generalist bee species.

The bumble bees (*Bombus*, Apidae) are a moderately large genus of bees comprising around 265 species globally in 15 subgenera (Arbetman et al., 2017; Cameron & Sadd, 2020). The genus is relatively young, arising some 25–40 million years ago in the Palearctic (Dehon et al., 2019; Hines, 2008). Excluding the fraction (10%) of socially parasitic species, all species form eusocial colonies that persist for several months, or even across multiple years in tropical species. As is expected from this long activity period (Waser et al., 1996), most bumble bees are generalist foragers (polylectic) with species collecting pollen from many different botanical families (Kleijn & Raemakers, 2008; Wood et al., 2019), even if individuals can be spatially and temporally specialised (Leonhardt & Blüthgen, 2012). Though predominantly a genus of generalists, the species *Bombus gerstaeckeri* breaks this pattern, as it is known to be a specialist (oligolectic) on plants of the genus *Aconitum* (Ranunculaceae, Gosselin et al., 2013), this specialisation all the more notable because it occurs in a group of otherwise polylectic species. Bumble bees therefore represent an ideal generalised lineage in which to investigate whether conserved pollen collection patterns exist.

There has been a long-running debate over patterns of pollen collection in bumble bees, as to whether certain species have inherently narrower and less flexible diets, and how this relates to their persistence in the face of global change (Goulson et al., 2005; Kleijn & Raemakers, 2008; Rasmont, 1988; Williams, 1985; Williams et al., 2007; Wood et al., 2019). Empirical studies documenting variation in dietary breadth have been geographically and phylogenetically limited in scope, and therefore insufficient to answer fully whether bumble bees display conserved patterns of pollen collection. Moreover, there has also been a long-running debate over the importance of bumble bee tongue length (as used here specifically referring to the sum of the lengths of the glossa and prementum) as a mechanism shaping the flowers that they visit and therefore their diets through morphological exclusion, competition and niche partitioning (Goulson et al., 2005; Harder, 1985; Heinrich, 1976; Inoue & Yokoyama, 2006; Inouye, 1978; Miller-Struttman et al., 2015). Bumble bee tongues show a high degree of interspecific variation, with short-tongued species in subgenera such as *Cullumanobombus* and *Pyrobombus* having average worker tongue lengths of 5–6 mm, and long-tongued species in subgenera such as *Megabombus* having

average worker tongue lengths of 12–13 mm (Arbetman et al., 2017), this variation in tongue length being independent of overall body size. As tongue length is strongly phylogenetically conserved in bumble bees (Arbetman et al., 2017), it would be a prime candidate to explain any similarly conserved dietary patterns, should they exist.

We address the question of conserved pollen collection patterns in bumble bees directly using the most complete quantitative dataset on bumble bee pollen diets assembled to date, in combination with a robust phylogeny of the genus. We hypothesise that bumble bees will show a conserved pattern of pollen use as seen in other bee lineages despite their generalised nature. We additionally test whether bumble bee tongue length is associated with any pollen collection patterns, and hypothesise that it will be given its role in structuring flower visitation in this genus.

2 | MATERIALS AND METHODS

2.1 | Pollen diet quantification

Bumble bee pollen diets were quantified following established methodologies (Kleijn & Raemakers, 2008; Wood et al., 2019; see Supporting Information Methods). Briefly, dried pollen balls were removed from selected pinned bumble bee specimens taken from museum and private collections to achieve the greatest possible taxonomic representation. The use of museum material is necessary for species such as *Bombus cullumanus* that have declined to extinction across large parts of their range (Williams et al., 2013), and therefore cannot easily be studied in contemporary landscapes. These balls were rehydrated, stained with fuchsin jelly, and pollen grains were identified using light microscopy, predominantly to genus. The proportion of each plant taxon was assessed, with taxa representing <2%–5% excluded (depending on identifier) as these may have arisen from contamination (Müller, 1996). These proportions were weighted by the overall size of the pollen ball relative to a full load, and weightings were used to calculate final dietary proportions. A total of 4,136 pollen loads from 58 bumble bee species belonging to 13 subgenera (representing approximately 93% of subgenera and 24% of species that collect pollen, Dataset S1, Dataset S2) from 34 countries were analysed (Table S1), 1,743 of which were published previously (Kleijn & Raemakers, 2008; Wood et al., 2019). Specimens for which label dates were available ($n = 4,089$) were collected between 1874 and 2020 (median = 1992). Data of capture were not considered or controlled for in this study, as there is no evidence to suggest that bumble bee diets change meaningfully over time (Kleijn & Raemakers, 2008). The most important museums were the Laboratory of Zoology collection, University of Mons (1,221 specimens, Mons, Belgium), the Naturalis Biodiversity Center (552 specimens, Leiden, the Netherlands), the J.B. Wallis/R.E. Roughley Museum of Entomology (369 specimens, Winnipeg, Canada), the Royal Belgian Institute of Natural Sciences (341 specimens, Brussels, Belgium), the Bavarian State Collection of Zoology (323 specimens, Munich, Germany), the A.J. Cook Arthropod Research Collection

(296 specimens, East Lansing, USA) and the Natural History Museum (239 specimens, London, UK). Full details are available in Dataset S2.

2.2 | Dietary metrics

Results were analysed at the botanical family level as this is the level at which most dietary specialisation occurs in insects (Forister et al., 2015), and to facilitate comparison between biogeographical regions where the same botanical families are present but are comprised of different constituent genera. Three major traits were investigated for a pattern of phylogenetic conservation: (a) dietary breadth, (b) diet dissimilarity and (c) collection from specific botanical families. To compare dietary breadth, results were standardised to produce a measure of the number of botanical families each species would be expected to collect in a sample size of 15 pollen loads (the smallest sample size, *B. haematurus*) using rarefaction. Rarefaction was conducted on pollen data (weighted percentage) after its transformation to complete pollen load equivalents (integer data) following Wood et al. (2019). The function *rarefy* from the *VEGAN* package (Oksanen et al., 2015) was used to calculate dietary breadth values based on the observed frequencies of collected botanical families, chosen at random without replacement 1,000 times. As this procedure is designed for integer data (e.g. the number of discrete visits to different flower species) and the pollen-load analysis data are non-integer (proportions of differently sized pollen loads), the pollen-load data were first transformed. The total number of pollen loads analysed for each species was multiplied by the proportion of each collected pollen. For example, across all samples, *B. fervidus* ($n = 66$) collected from 13 botanical families. In total, 63.8% of its pollen was collected from Fabaceae, giving 40.1 'complete' pollen-load equivalents, that is, in 66 unique *B. fervidus* foraging bouts for pollen, 40 would functionally have been to Fabaceae. This value was calculated for each botanical family collected by each bumble bee species, and then these values were all multiplied by 10 and rounded to the nearest whole number to give an integer equivalent used in the rarefaction procedure. For *B. fervidus*, when rarefied to a sample size of 15 pollen loads, this gives a comparable diet breadth of 10.513 botanical families.

For dietary dissimilarity, pairwise differences in dietary composition (Bray–Curtis dissimilarity, using the transformed integer dataset for rarefaction) were calculated between each species pair using the *vegdist* function in the (*VEGAN* package). Scores can range between '1' (= perfectly dissimilar) and '0' (= perfectly similar). The clearest example of dissimilarity can be seen with *B. gerstaeckeri*, which collected all of its pollen from Ranunculaceae. It therefore received a pairwise dissimilarity score of '1.000' with all bumble bee species that did not collect any Ranunculaceae. The greatest example of similarity can be seen between *B. armeniacus* and *B. distinguendus* that collected from relatively few botanical families with a high degree of overlap, Fabaceae plus Boraginaceae accounting for 75.9% of the diet of *B. armeniacus*, and 89.4% of the diet of *B. distinguendus*. This generated a pairwise dissimilarity score of '0.166', indicating high

dietary similarity. These pairwise differences were averages for each species (57 comparisons per species) to produce an average dissimilarity score.

Collection from specific botanical families was calculated as a simple percentage across the entire diet, for example for Fabaceae, *B. affinis* collected 11.4% of its pollen from this botanical family, *B. alagesianus* collected 47.9% and so on. This was calculated for the top 10 most important botanical families as defined below.

2.3 | Data analysis

Dietary metrics were tested for phylogenetic signal. We used the R package PHYTOOLS (Revell, 2012) to estimate and assess the level of significance of the K statistic measuring the phylogenetic signal of chosen traits (standardised dietary breadth, averaged dissimilarity, percentage of specific botanical families collected) by comparing the observed signal in each trait to the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg et al., 2003). This estimation was based on the maximum clade credibility tree obtained from the most updated Bayesian phylogenetic analysis performed by Cameron et al. (2007) for the genus *Bombus*, including all but two of the species in our dataset (*B. cullumanus* and *B. bisiculus*, sequences not available).

The composition of bumble bee pollen diets was visualised using a non-metric multidimensional scaling (NMDS) plot with the *ordiplot* function from the VEGAN package (Bray–Curtis dissimilarity). The impact of subgenus was tested using permutational multivariate analysis of variance (PERMANOVA) tests with the function *adonis* (VEGAN package). Only subgenera with three or more representatives were included. Two analyses were run, with and without the highly aberrant *B. gerstaeckeri*. For the full dataset, the most important botanical families contributing to differences in pollen-load composition (dissimilarity) among individual species were identified using similarity percentage (SIMPER) analyses with the *simper* function (VEGAN package). Because the SIMPER analysis requires categorical groupings, bumble bee species were sorted by overall dissimilarity score, with half ($n = 29$) placed in a 'high dissimilarity' group, and half ($n = 29$) in a 'low dissimilarity' group. The top 10 botanical families contributing most to the difference in dissimilarity scores after SIMPER analysis were tested for phylogenetic signal. The relationship between bumble bee tongue length and dietary metrics was tested using linear models, or in the case of dietary dissimilarity, Spearman's rank correlation as the response variable could not be transformed to normality. Bumble bee tongue length values were taken from a literature compilation (Arbetman et al., 2017), with values available for 41 of the 58 species studied here. Phylogenetic least squares regression analyses were conducted using the package APE (Paradis & Schliep, 2019) to ensure that observed relationships with tongue length were not driven by phylogenetic proximity. To ensure that tongue length was not confounded by bumble bee body size, this was independently tested for phylogenetic signal and correlation

with tongue length (see Supporting Information Methods). All analyses were conducted in R version 3.6.3.

3 | RESULTS

Studied bumble bee species were widely polylectic, collecting from 60 botanical families, with a typical bumble bee species collecting 9.9 ± 0.6 botanical families in 15 analysed pollen loads after standardisation. However, standardised dietary breadth showed no phylogenetic signal (Blomberg's $K = 0.248$, $p = 0.114$, Figure S1). Instead, dietary dissimilarity was associated with a phylogenetic signal ($K = 0.307$, $p = 0.028$), with the subgenera *Mendacibombus*, *Bombias* and *Thoracobombus* showing a conserved trend of similar diets with a high Fabaceae content (Figure 1).

Dietary composition showed structuring by subgenus, with *Thoracobombus* clearly collecting from a restricted subset of the botanical families used by the subgenus *Pyrobombus*, which occupied the widest variety of dietary niches (PERMANOVA, $F_{6,41} = 2.19$, $p = 0.001$, Figure S2). All other subgenera were intermediate between the two. Including the aberrant *B. gerstaeckeri*, which is a specialist of *Aconitum* (Ranunculaceae), did not change the overall relationship, but greatly distorted the overall pattern ($F_{6,42} = 2.14$, $p = 0.003$, Figure S3). SIMPER analysis between the high and low dissimilarity groups showed that Fabaceae explained the largest proportion of variation between the groups (39.7%), with members of the low dissimilarity group collecting on average twice as much Fabaceae as the high dissimilarity group (Table 1). The next highest contributor, Asteraceae, explained just 7.1% of variation. When these top 10 botanical families contributing most to overall variation between the two groups were tested individually, the percentage of pollen collected from Fabaceae ($K = 0.440$, $p < 0.001$), Lamiaceae ($K = 0.327$, $p = 0.022$), Rosaceae ($K = 0.380$, $p = 0.019$) and Hypericaceae ($K = 0.350$, $p = 0.014$) were all associated with a phylogenetic signal (full results Table S2).

Collection of Fabaceae explained much of the overall variation in dietary dissimilarity scores among individual species ($R^2 = 0.682$), with the next highest, Ranunculaceae ($R^2 = 0.227$), explaining substantially less and showing no phylogenetic signal ($K = 0.217$, $p = 0.488$, full results Table S2). In contrast, Lamiaceae ($R^2 = 0.003$), Rosaceae ($R^2 = 0.022$) and Hypericaceae ($R^2 = 0.003$) explain very little to none of this variation despite their phylogenetic signal. The use of Fabaceae pollen therefore largely determines overall dietary dissimilarity, with species with the lowest dissimilarity scores preferentially using this family, and species with the greatest dissimilarity scores favouring a wide range of different botanical families (Table 2).

There was no relationship between tongue length and dietary breadth (LM, $t_{1,39} = 1.252$, $p = 0.218$, $R^2 = 0.039$, Figure 2a), but both a lower dietary dissimilarity score (Spearman's $Rho = 0.602$, $p < 0.001$, $R^2 = 0.209$, Figure 2b) and higher collection of pollen from Fabaceae (LM, $t_{1,39} = 3.121$, $p = 0.003$, $R^2 = 0.200$, Figure 2c) were associated with longer tongue length. However, both the results for

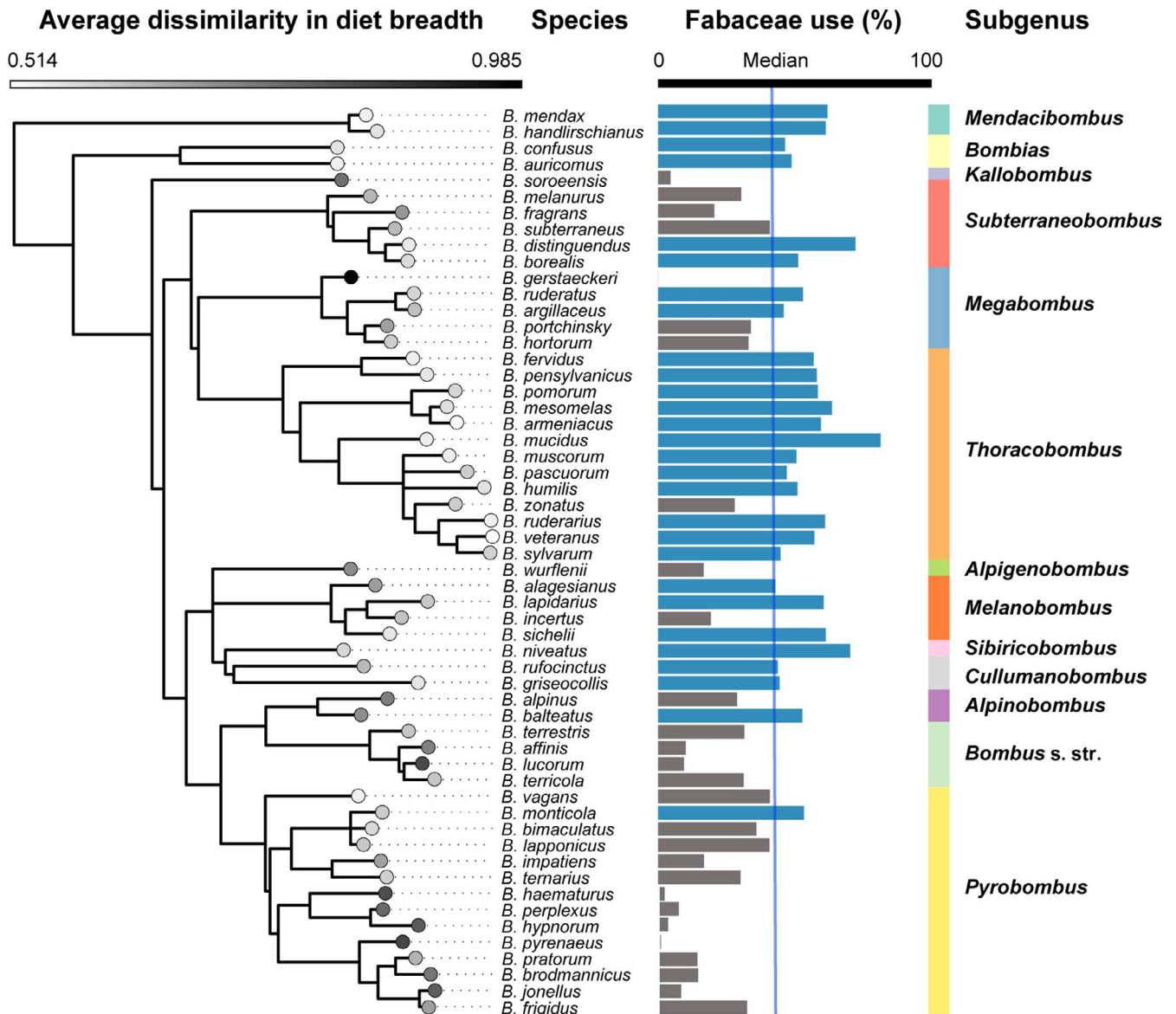


FIGURE 1 Average pollen diet composition dissimilarity (Bray–Curtis dissimilarity, relative to diets of other bumble bee species, 0 = completely similar, 1 = completely dissimilar) mapped onto the bumble bee phylogeny. For each species, the percentage of the diet composed of Fabaceae is also indicated

dietary dissimilarity (PGLS, $t_{1,39} = 1.357$, $p = 0.183$) and collection of Fabaceae (PGLS, $t_{1,39} = 0.859$, $p = 0.395$) were non-significant after phylogenetic correction. Body size showed a weak positive correlation with tongue length, but no phylogenetic signal and no association with any of the dietary metrics (see Supporting Information Results).

4 | DISCUSSION

Bumble bees show a phylogenetically conserved pattern of pollen collection, not in the overall breadth of their diet, but in the composition of collected botanical families. This is most clearly expressed in the *Mendacibombus*, *Bombias* and *Thoracobombus* lineages, these all displaying a strong association with pollen from the Fabaceae

which dominates their diets. Although bumble bees are some of the most hyper-generalised of all insect herbivores, regularly collecting from more than 10 botanical families (Forister et al., 2015), these conserved patterns show that they are still subject to dietary restrictions that constrain their foraging choices as seen in solitary and more specialised bee lineages (Dellicour et al., 2014; Haider et al., 2014; Müller, 1996; Patiny et al., 2007; Sedivy et al., 2008; Sipes & Tepedino, 2005).

The conservation of the pollen diet shown by these solitary bee lineages (*Melitta*, *Osmia* subgenus *Osmia*, Anthidini, Rophitinae, *Chelostoma*, *Didasia*, respectively) differs from that seen in bumble bees by its much greater incidence of specialisation and host switching. Though clusters of related species feeding on the same host family make for a clear pattern, when host switching occurs it can be abrupt. For example, three closely related specialists *Melitta*

TABLE 1 Similarity percentage (SIMPER) analysis comparing the composition of pollen loads collected by high ($n = 29$) and low dissimilarity ($n = 29$) bumble bee species. Only the top 10 botanical families contributing the most to overall variation are included

Botanical family	Use by high dissimilarity group (%)	Use by low dissimilarity group (%)	Average differential (% points)	Contribution to overall variation (%)
Fabaceae	28.9	58.7	29.8	39.7
Asteraceae	9.9	5.7	4.2	7.1
Lamiaceae	8.4	7.0	1.4	6.8
Ericaceae	8.1	2.9	5.1	6.3
Rosaceae	6.3	4.4	2.0	5.6
Boraginaceae	6.0	4.6	1.4	5.2
Orobanchaceae	5.6	3.9	1.7	4.9
Campanulaceae	7.2	0.6	6.7	4.5
Ranunculaceae	2.1	0.9	1.2	2.2
Hypericaceae	1.5	1.9	0.4	2.1

Species	Subgenus	Dissimilarity score	Dominant botanical family	Percentage dominance
(a)				
<i>B. gerstaeckeri</i>	<i>Megabombus</i>	0.985	Ranunculaceae	100.0
<i>B. pyrenaicus</i>	<i>Pyrobombus</i>	0.845	Campanulaceae	38.4
<i>B. lucorum</i>	<i>Bombus</i> s. str.	0.844	Apiaceae	31.4
<i>B. haematurus</i>	<i>Pyrobombus</i>	0.831	Lamiaceae	41.7
<i>B. hypnorum</i>	<i>Pyrobombus</i>	0.803	Rosaceae	36.2
<i>B. jonellus</i>	<i>Pyrobombus</i>	0.795	Ericaceae	62.2
<i>B. perplexus</i>	<i>Pyrobombus</i>	0.775	Rosaceae	27.8
<i>B. soroensis</i>	<i>Kallobombus</i>	0.763	Campanulaceae	47.2
<i>B. cullumanus</i>	<i>Cullumanobombus</i>	0.754	Asteraceae	45.2
<i>B. brodmannicus</i>	<i>Pyrobombus</i>	0.750	Boraginaceae	36.9
(b)				
<i>B. armeniacus</i>	<i>Thoracobombus</i>	0.514	Fabaceae	66.5
<i>B. veteranus</i>	<i>Thoracobombus</i>	0.516	Fabaceae	63.9
<i>B. mendax</i>	<i>Mendacibombus</i>	0.537	Fabaceae	69.2
<i>B. auricomus</i>	<i>Bombias</i>	0.537	Fabaceae	54.6
<i>B. ruderarius</i>	<i>Thoracobombus</i>	0.538	Fabaceae	68.2
<i>B. vagans</i>	<i>Pyrobombus</i>	0.538	Fabaceae	45.5
<i>B. fervidus</i>	<i>Thoracobombus</i>	0.539	Fabaceae	63.8
<i>B. muscorum</i>	<i>Thoracobombus</i>	0.544	Fabaceae	56.6
<i>B. distinguendus</i>	<i>Subterraneobombus</i>	0.545	Fabaceae	80.6
<i>B. bisiculus</i>	<i>Melanobombus</i>	0.547	Fabaceae	73.0

TABLE 2 Dominant botanical family in collected pollen (% of the diet) for the top 10 bumble bee species with the (a) most and (b) least dissimilar dietary scores (Bray–Curtis dissimilarity, 0 = perfectly similar, 1 = perfectly dissimilar)

leporina, *M. tricincta* and *M. nigricans* specialise on unrelated flowers with divergent morphology, namely Fabaceae (zygomorphic), *Odontites* (Orobanchaceae, sympetalous) and *Lythrum* (Lythraceae, actinomorphic, Vanderplanck et al., 2017). In contrast, the generalist nature of bumble bees means that major hosts like Fabaceae are usually still present in the diets of clades that have moved onto other resources, such as the *Pyrobombus* which still collect an average of 23.7% of their pollen from this family despite clearly having a low overall affinity with it. Bumble bees most clearly differ from

these previously studied lineages by their sociality and flight period length, the two going hand in hand as the production of a worker caste necessitates an extended period of activity. This long flight period means that specialising on a single resource is not a viable strategy (Waser et al., 1996), unless said resource has an extremely long flowering period, and even then it may be necessary to skip the worker generation to reduce overall flight period length as seen in *B. gerstaeckeri* (Ponchau et al., 2006). This long flight period may be the reason that when bumble bee clades move away from Fabaceae,

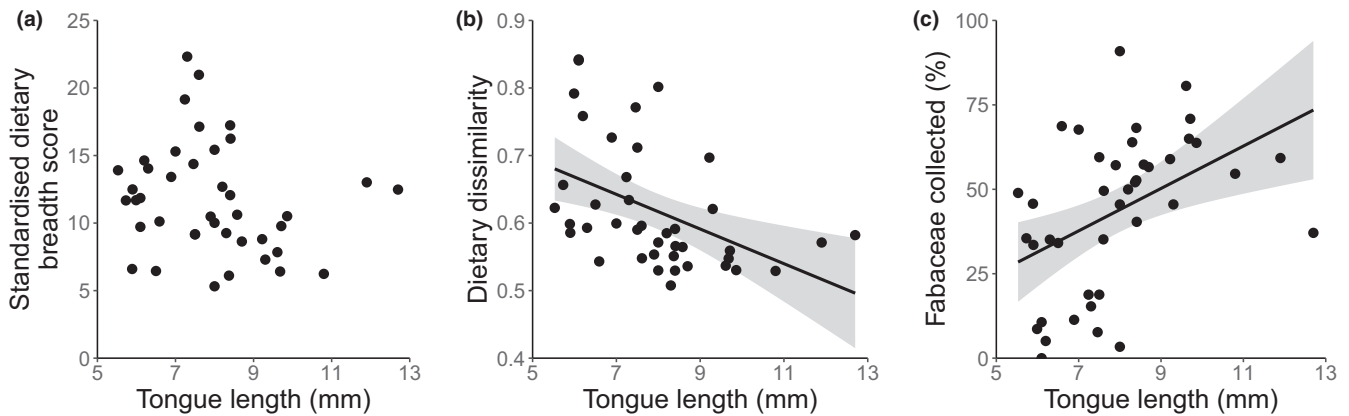


FIGURE 2 Relationship between average worker tongue length (mm) and (a) standardised dietary breadth score, (b) dietary dissimilarity score and (c) average percentage of Fabaceae collected. Lines represent significant relationships with 95% confidence intervals, indicated by grey shaded areas

they do so less abruptly, and this reduced collection remains visible in their pollen collection patterns.

While overall constrained pollen collection patterns in bumble bees are apparent, the specific mechanisms governing their existence require discussion, most clearly the phylogenetically conserved trait of tongue length (Arbetman et al., 2017). Though well established, much of the work on tongue length driving bumble bee dietary niches has focused on the collection of nectar (Harder, 1985; Heinrich, 1976; Inouye, 1978), and not pollen, as the tongue itself is not directly involved in pollen removal (as opposed to adaptations present in other bee lineages, e.g. Müller, 2006). Why then should tongue length shape pollen collection? Bumble bees have high energetic (calorific) needs and are sensitive to floral nectar rewards, making foraging decisions based on the profitability of different plant species (Heinrich, 1979; Inouye, 1978), which, in turn, is affected by flower handling efficiency that is strongly influenced by tongue length (Harder, 1983). It has consequently been argued that, as long-tongued bumble bees collect pollen while nectar foraging, they out-compete short-tongued bees on flowers with deep corollae and force these species to visit plants with short corollae instead (Heinrich, 1976). The concurrent collection of pollen and nectar therefore provides a mechanistic explanation as to how tongue length could determine pollen foraging patterns in bumble bees. However, the lack of a relationship between tongue length and foraging patterns after correction for evolutionary distance suggests that these relationships are being driven by phylogenetic relatedness rather than tongue length per se.

A question remains, however, why is the botanical family Fabaceae so important? The Fabaceae itself is a hyper-diverse plant lineage with almost 20,000 species, the third largest botanical family this metric, and they occur in almost all habitats worldwide from tropical forests to Mediterranean, desert and temperate regions, and to high latitudes and altitudes (Legume Phylogeny Working Group (LPWG), 2017). Bumble bees evolved some 25–40 million years ago in the Palearctic during a period of rapid global cooling (Dehon et al., 2019; Hines, 2008), and as such all major botanical lineages including Fabaceae would nominally have been available to

them (Wikström et al., 2001). The large majority of bumble bee species are found in areas with distinct seasons that render year-round colony development impossible. This comparatively short season may have favoured the use of the most rewarding pollen sources with high protein content such as members of the Fabaceae (Hanley et al., 2008), or a high protein to lipid ratio (Vaudo et al., 2016) to achieve the fastest possible colony growth (Moerman et al., 2016). Though plausible, this idea forms at most a hypothesis (Smith, 2016), as it is ultimately not possible to demonstrate why bumble bees became strongly associated with Fabaceae as opposed to any other botanical family available to them during their evolution. Alternative hypotheses may be that Fabaceae was favoured due to its abundance in cold environments and because they have high and reliable provision of both pollen and nectar. It should be noted that though the family itself is very diverse, bumble bees do not show an association with most Fabaceae lineages, instead specifically favouring those with a symmetrical (zygomorphic) flower structure, these being found predominantly within the Papilionoideae (Legume Phylogeny Working Group (LPWG), 2017). This flower shape necessitates animal pollination (e.g. the explosive mechanism used by *Desmodium*, Alemán et al., 2014) and may be involved with the link between this lineage and bumble bees, but more direct evidence is lacking. Future experimental work conducted on conserved bumble bee lineages may allow for hypotheses arising from these ideas to be tested more robustly, as to date most manipulative studies have been conducted on the derived lineages *Bombus* s. str. and *Pyrobombus* that show no meaningful association with Fabaceae (Figure 1).

The restricted pollen foraging choices of bumble bees have implications for their continued persistence and successful conservation. A global change affecting the availability of a particular resource will collectively affect any phylogenetically related group constrained to using that resource. Inversely, a phylogenetically related group that lacks a conserved dietary pattern means that it is less likely to be collectively affected because members pursue an inherently wider variety of strategies. In bumble bees, these two patterns are, respectively, well illustrated by the subgenera *Thoracobombus* (all 13 studied species favouring Fabaceae) and *Pyrobombus* (the

14 studied species individually favouring seven different botanical families), and these two subgenera were identified as being the most and least over-represented subgenera for declining species globally (*Thoracobombus*, 64% declining, *Pyrobombus*, 6% declining; Arbetman et al., 2017).

Bumble bees sharing a conserved niche may not be more likely to decline per se, and dietary niche may not be the principal predictor of decline, as this is not seen in global analyses (Arbetman et al., 2017). Indeed, bumble bees face other major threats to their populations such as global warming and pathogens (Cameron & Sadd, 2020; Rasmont et al., 2015). However, the fact that meaningful constraints exist has implications for species dependent on specific resources. The loss of preferred host plants is a key driver of bee species decline (Scheper et al., 2014), and the impact of such a loss cannot be avoided by species lacking dietary flexibility. The inflexible strategy pursued by lineages such as the *Thoracobombus* is therefore more likely to result in a more uniform decline should their preferred host rapidly decrease in abundance, as occurred to Fabaceae during the 20th century in Europe and North America (Goulson et al., 2005; Rasmont, 1988; Scheper et al., 2014; Wood et al., 2019). Fabaceae are a botanical family of enormous commercial value, leading to the movement of species around the world. Industrial Fabaceae cultivation in North America relied heavily on the use of non-native species from the Palearctic, many of which are now invasive. The role that non-native species play in forming and shaping the dietary niches and ranges of North American bumble bees should be further investigated. In contrast to the patterns seen in these seemingly inflexible bumblebee lineages, the evolutionary shift demonstrated here from Fabaceae onto a wider range of host families may have preadapted members of derived bumble bee clades like *Pyrobombus* to the environmental changes caused by the Anthropocene, and made it less likely for them to be collectively affected by the decline of any particular resource.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.



AUTHORS' CONTRIBUTIONS

T.J.W. and G.G. conceived the study; all authors provided the specimens; T.J.W., D.K. and M.K. carried out pollen identification and quantification; T.J.W. and G.G. ran the analyses and T.J.W. led writing of the manuscript; all authors contributed critically to the writing process.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.63xsj3v2g> (Wood et al., 2021).

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REFERENCES

- Alemán, M., Figueroa-Fleming, T., Etcheverry, Á., Sühling, S., & Ortega-Baes, P. (2014). The explosive pollination mechanism in Papilionoideae (Leguminosae): An analysis with three *Desmodium* species. *Plant Systematics and Evolution*, 300, 177–186. <https://doi.org/10.1007/s00606-013-0869-8>
- Arbetman, M., Gleiser, G., Morales, C., Williams, P., & Aizen, M. A. (2017). Global decline of bumblebees is phylogenetically structured and inversely related to species range size and pathogen incidence. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20170204. <https://doi.org/10.1098/rspb.2017.0204>
- Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, 57, 717–745. <https://doi.org/10.1111/j.0014-3820.2003.tb00285.x>
- Cameron, S. A., Hines, H. M., & Williams, P. H. (2007). A comprehensive phylogeny of the bumble bees (*Bombus*). *Biological Journal of the Linnean Society*, 91, 161–188. <https://doi.org/10.1111/j.1095-8312.2007.00784.x>
- Cameron, S. A., & Sadd, B. M. (2020). Global trends in bumble bee health. *Annual Review of Entomology*, 65, 209–232. <https://doi.org/10.1146/annurev-ento-011118-111847>
- Dafni, A. (1984). Mimicry and deception in pollination. *Annual Review of Ecology and Systematics*, 15, 259–278. <https://doi.org/10.1146/annurev.es.15.110184.001355>
- Dehon, M., Engel, M. S., Gérard, M., Aytakin, A. M., Ghisbain, G., Williams, P. H., Rasmont, P., & Michez, D. (2019). Morphometric analysis of fossil bumble bees (Hymenoptera, Apidae, Bombini) reveals their taxonomic affinities. *ZooKeys*, 891, 71–118. <https://doi.org/10.3897/zookeys.891.36027>
- Dellicour, S., Lecocq, T., Kuhlmann, M., Mardulyn, P., & Michez, D. (2014). Molecular phylogeny, biogeography, and host plant shifts in the bee genus *Melitta* (Hymenoptera: Anthophila). *Molecular Phylogenetics and Evolution*, 70, 412–419. <https://doi.org/10.1016/j.ympev.2013.08.013>
- Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution*, 18, 586–608. <https://doi.org/10.1111/j.1558-5646.1964.tb01674.x>
- Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T., Cizek, L., Coley, P. D., Dem, F., Diniz, I. R., Drozd, P., Fox, M., Glassmire, A. E., Hazen, R., Hrcek, J., Jahner, J. P., Kaman, O., Kozubowski, T. J., Kursar, T. A., ... Dyer, L. A. (2015). The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 442–447. <https://doi.org/10.1073/pnas.1423042112>
- Gosselin, M., Michez, D., Vanderplanck, M., Roelants, D., Glauser, G., & Rasmont, P. (2013). Does *Aconitum septentrionale* chemically protect floral rewards to the advantage of specialist bumblebees? *Ecological Entomology*, 38, 400–407. <https://doi.org/10.1111/een.12032>

- Goulson, D., Hanley, M., Darvill, B., Ellis, J. S., & Knight, M. E. (2005). Causes of rarity in bumblebees. *Biological Conservation*, 122, 1–8. <https://doi.org/10.1016/j.biocon.2004.06.017>
- Haider, M., Dorn, S., Sedivy, C., & Müller, A. (2014). Phylogeny and floral hosts of a predominantly pollen generalist group of mason bees (Megachilidae: Osmiini). *Biological Journal of the Linnean Society*, 111, 78–91. <https://doi.org/10.1111/bij.12186>
- 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. <https://doi.org/10.1111/j.1365-2435.2008.01415.x>
- Harder, L. D. (1983). Flower handling efficiency of bumble bees: Morphological aspects of probing time. *Oecologia*, 57, 274–280. <https://doi.org/10.1007/BF00379591>
- Harder, L. D. (1985). Morphology as a predictor of flower choice by bumble bees. *Ecology*, 66, 198–210. <https://doi.org/10.2307/1941320>
- Hardy, N. B., Kaczkvinsky, C., Bird, G., & Normark, B. B. (2020). What we don't know about diet-breadth evolution in herbivorous insects. *Annual Review of Ecology, Evolution, and Systematics*, 51, 103–122. <https://doi.org/10.1146/annurev-ecolsys-011720-023322>
- Heinrich, B. (1976). Bumblebee Foraging and the Economics of Sociality: How have bumblebees evolved to use a large variety of flowers efficiently? Individual bees have specialized behavioural repertoires, and the colony, collectively, can harvest food from many different resources. *American Scientist*, 64, 384–395.
- Heinrich, B. (1979). *Bumblebee economics*. Harvard University Press.
- Hines, H. M. (2008). Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: *Bombus*). *Systematic Biology*, 57, 58–75. <https://doi.org/10.1080/10635150801898912>
- Hunter, M. (2016). *The phytochemical landscape: Linking trophic interactions and nutrient dynamics*. Princeton University Press. 376 pp.
- Inoue, M. N., & Yokoyama, J. (2006). Morphological variation in relation to flower use in bumblebees. *Entomological Science*, 9, 147–159. <https://doi.org/10.1111/j.1479-8298.2006.00162.x>
- Inouye, D. W. (1978). Resource partitioning in bumblebees: Experimental studies of foraging behavior. *Ecology*, 59, 672–678. <https://doi.org/10.2307/1938769>
- Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, 21, 243–273. <https://doi.org/10.1146/annurev.es.21.110190.001331>
- Janz, N., & Nylin, S. (1998). Butterflies and plants: A phylogenetic study. *Evolution*, 52, 486–502. <https://doi.org/10.2307/2411084>
- Kleijn, D., & Raemakers, I. (2008). A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology*, 89, 1811–1823. <https://doi.org/10.1890/07-1275.1>
- Legume Phylogeny Working Group (LPWG). (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon*, 66, 44–77. <https://doi.org/10.12705/661.3>
- Leonhardt, S. D., & Blüthgen, N. (2012). The same, but different: Pollen foraging in honeybee and bumblebee colonies. *Apidologie*, 43, 449–464. <https://doi.org/10.1007/s13592-011-0112-y>
- Miller-Struttmann, N. E., Geib, J. C., Franklin, J. D., Kevan, P. G., Holdo, R. M., Ebert-May, D., Lynn, A. M., Kettenbach, J. A., Hedrick, E., & Galen, C. (2015). Functional mismatch in a bumble bee pollination mutualism under climate change. *Science*, 349, 1541–1544. <https://doi.org/10.1126/science.aab0868>
- Moerman, R., Vanderplanck, M., Roger, N., Declèves, S., Wathelet, B., Rasmont, P., Fournier, D., & Michez, D. (2016). Growth rate of bumble bee larvae is related to pollen amino acids. *Journal of Economic Entomology*, 109, 25–30. <https://doi.org/10.1093/jee/tov279>
- Moldenke, A. R. (1979). The role of host-plant selection in bee speciation processes. *Phytologia*, 43, 433–460. <https://doi.org/10.5962/bhl.part.27764>
- Müller, A. (1996). Host-plant specialization in Western Palearctic Anthidine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs*, 66, 235–257. <https://doi.org/10.2307/2963476>
- Müller, A. (2006). Unusual host plant of *Hoplitis pici*, a bee with hooked bristles on its mouthparts (Hymenoptera: Megachilidae: Osmiini). *European Journal of Entomology*, 103, 497–500. <https://doi.org/10.14411/eje.2006.064>
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2015). *Vegan: Community ecology package*. R package version 2.3–2.
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Paradis, R., & Schliep, P. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35, 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Patiny, S., Michez, D., & Danforth, B. N. (2007). Phylogenetic relationships and host-plant evolution within the basal clade of Halictidae (Hymenoptera, Apoidea). *Cladistics*, 24, 255–269. <https://doi.org/10.1111/j.1096-0031.2007.00182.x>
- Ponchau, O., Iserbyt, S., Verhaeghe, J.-C., & Rasmont, P. (2006). Is the caste-ratio of the oligolectic bumblebee *Bombus gerstaeckeri* Morawitz (Hymenoptera: Apidae) biased to queens? *Annales de la Société Entomologique de la France*, 42, 207–214. <https://doi.org/10.1080/00379271.2006.10700624>
- Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P. D., Howlett, B. G., Winfree, R., Cunningham, S. A., Mayfield, M. M., Arthur, A. D., Andersson, G. K. S., Bommarco, R., Brittain, C., Carvalheiro, L. G., Chacoff, N. P., Entling, M. H., Foully, B., Freitas, B. M., Gemmill-Herren, B., Ghazoul, J., ... Woyciechowski, M. (2016). Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 146–151. <https://doi.org/10.1073/pnas.1517092112>
- Rasmont, P. (1988). *Monographie écologique et zoogéographique des bourdons de France et de Belgique (Hymenoptera, Apidae, Bombinae)*. Ph.D. thesis. Faculté des Sciences Agronomiques de l'Etat.
- Rasmont, P., Franzen, M., Lecocq, T., Harpke, A., Roberts, S., Biesmeijer, K., Castro, L., Cederberg, B., Dvorak, L., Fitzpatrick, U., Gonthier, Y., Haubruge, E., Mahe, G., Manino, A., Michez, D., Neumayer, J., Odegaard, F., Paukkunen, J., Pawlikowski, T., ... Schweiger, O. (2015). Climatic risk and distribution atlas of European bumblebees. *Biodiversity and Ecosystem Risk Assessment*, 10, 1–246. <https://doi.org/10.3897/biorisk.10.4749>
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Robertson, C. (1925). Heterotrophic bees. *Ecology*, 6, 412–436. <https://doi.org/10.2307/1929107>
- Scheper, J., Reemer, M., van Kats, R., Ozinga, W. A., van der Linden, G. T. J., Schaminée, J. H. J., Siepel, H., & Kleijn, D. (2014). Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in The Netherlands. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 17552–17557. <https://doi.org/10.1073/pnas.1412973111>
- Sedivy, C., Praz, C. J., Müller, A., Widmer, A., & Dorn, S. (2008). Patterns of host-plant choice in bees of the genus *Chelostoma*: The constraint hypothesis of host-range evolution in bees. *Evolution*, 62, 2487–2507. <https://doi.org/10.1111/j.1558-5646.2008.00465.x>
- Sipes, S. D., & Tepedino, V. J. (2005). Pollen-host specificity and evolutionary patterns of host switching in a clade of specialist bees (Apoidea: *Diadasia*). *Biological Journal of the Linnean Society*, 86, 487–505. <https://doi.org/10.1111/j.1095-8312.2005.00544.x>
- Smith, R. J. (2016). Explanations for adaptations, just-so-stories, and limitations on evidence in evolutionary biology. *Evolutionary Anthropology*, 25, 276–287. <https://doi.org/10.1002/evan.21495>

- Steffan, S. A., Dharampal, P. S., Danforth, B. N., Gaines-Day, H. R., Takizawa, Y., & Chikaraishi, Y. (2019). Omnivory in bees: Elevated trophic positions among all major bee families. *The American Naturalist*, 194, 414–421. <https://doi.org/10.1086/704281>
- Vanderplanck, M., Vereecken, N. J., Grumiau, L., Esposito, F., Lognay, G., Wattiez, R., & Michez, D. (2017). The importance of pollen chemistry in evolutionary host shifts of bees. *Scientific Reports*, 7, 43058. <https://doi.org/10.1038/srep43058>
- Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016). Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E4035–E4042. <https://doi.org/10.1073/pnas.1606101113>
- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043–1060. <https://doi.org/10.2307/2265575>
- Westerkamp, C. (1996). Pollen in bee–flower relations some considerations on Melittophily. *Botanica Acta*, 109, 325–332. <https://doi.org/10.1111/j.1438-8677.1996.tb00580.x>
- Wikström, N., Savolainen, V., & Chase, M. W. (2001). Evolution of the angiosperms: Calibrating the family tree. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 2211–2220. <https://doi.org/10.1098/rspb.2001.1782>
- Williams, P. H. (1985). *On the distribution of bumble bees (Hymenoptera: Apidae) with particular regard to patterns within the British Isles*. Department of Applied Biology, University of Cambridge.
- Williams, P. H., Araújo, M. B., & Rasmont, P. (2007). Can vulnerability among British bumblebee (*Bombus*) species be explained by niche position and breadth? *Biological Conservation*, 138, 493–505. <https://doi.org/10.1016/j.biocon.2007.06.001>
- Williams, P. H., Byvaltsev, A., Sheffield, C., & Rasmont, P. (2013). *Bombus cullumanus* – An extinct European bumblebee species? *Apidologie*, 44, 121–132. <https://doi.org/10.1007/s13592-012-0161-x>
- Wood, T. J., Ghisbain, G., Rasmont, P., Kleijn, D., Raemakers, I., Praz, C., Killewald, M., Gibbs, J., Bobiwash, K., Boustani, M., Martinet, B., & Michez, D. (2021). Data from: Global patterns in bumble bee pollen collection show phylogenetic conservation of diet. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.63xsj3v2g>
- Wood, T. J., Gibbs, J., Graham, K. K., & Isaacs, R. (2019). Narrow pollen diets are associated with declining Midwestern bumble bee species. *Ecology*, 100, e02697. <https://doi.org/10.1002/ecy.2697>

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