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Pollinator or pedigree: which factors determine the evolution of pollen nutrients?

Fabian A. Ruedenauer¹ · Johannes Spaethe² · Casper J. van der Kooi³ · Sara D. Leonhardt¹ 

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Abstract

A prime example of plant–animal interactions is the interaction between plants and pollinators, which typically receive nectar and/or pollen as reward for their pollination service. While nectar provides mostly carbohydrates, pollen represents the main source of protein and lipids for many pollinators. However, the main function of pollen is to carry nutrients for pollen tube growth and thus fertilization. It is unclear whether pollinator attraction exerts a sufficiently strong selective pressure to alter the nutritional profile of pollen, e.g., through increasing its crude protein content or protein-to-lipid ratio, which both strongly affect bee foraging. Pollen nutritional quality may also be merely determined by phylogenetic relatedness, with pollen of closely related plants showing similar nutritional profiles due to shared biosynthetic pathways or floral morphologies. Here, we present a meta-analysis of studies on pollen nutrients to test whether differences in pollen nutrient contents and ratios correlated with plant insect pollinator dependence and/or phylogenetic relatedness. We hypothesized that if pollen nutritional content was affected by pollinator attraction, it should be different (e.g., higher) in highly pollinator-dependent plants, independent of phylogenetic relatedness. We found that crude protein and the protein-to-lipid ratio in pollen strongly correlated with phylogeny. Moreover, pollen protein content was higher in plants depending mostly or exclusively on insect pollination. Pollen nutritional quality thus correlated with both phylogenetic relatedness and pollinator dependency, indicating that, besides producing pollen with sufficient nutrients for reproduction, the nutrient profile of zoophilous plants may have been shaped by their pollinators' nutritional needs.

Keywords Foraging · Nutrition · Meta-analysis · Plant–insect interactions · Pollen quality · Pollination · Resource use

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Introduction

Interactions between organisms shape our environment and ecological communities, and drive ecosystem functions (Jones et al. 1996). One prominent example is the interaction between animals and plants, which is typically driven by resource use, as many animal species rely on plants for meeting their nutritional and/or protective needs (Berenbaum et al. 1986; Bernays 1989). Some plants provide chemically attractive rewards to attract partners, such as pollinators or seed dispersers (Waser 2006). In addition to non-nutritional floral factors such as color and scent (McCall and Primack 1992; van der Kooi et al. 2019b), reward nutritional quality can strongly affect the community of flower-visiting animals (Petanidou et al. 2006). For example, bees (Camazine and Sneyd 1991; Nicholls and Hempel de Ibarra 2016; Ruedenauer et al. 2016; Somme et al. 2015), butterflies (Lewis 1986), and hummingbirds (Stiles 1976) appear to select

plant species based on differences in the nutritional quality of rewards.

Pollen is a nutritionally diverse and highly valuable reward for pollinators, because it provides protein, fat, carbohydrates, sterols, and various micronutrients (Roulston and Cane 2000). Due to its valuable nutritional quality, some pollinators, such as bees, exclusively rely on pollen for reproduction and survival (e.g., Baidya et al. 1993; Haydak 1970; Loper and Berdel 1980; Saffari et al. 2010). These pollinators consequently need to find pollen that meet their nutritional needs, which may result in foraging choices and thus visitation patterns that are strongly, if not exclusively, determined by pollen nutritional quality. For example, bumblebees can assess the nutritional quality of pollen and preferentially forage on plants with pollen of high protein (and low lipid) content, while other (combinations of) pollen nutrients reduce the number of certain flower visitors (Kitajima and Nieh 2009; Leonhardt and Blüthgen 2012; Ruedenauer et al. 2015, 2016; Somme et al. 2015; Vaudo et al. 2016b). Thus, akin to nectar, pollen nutritional quality can significantly affect the spectrum of flower visitors. Pollinator foraging choices may in turn shape the nutritional composition of pollen of those plants that are highly dependent on animal pollination. Such plants may benefit from increased visitation—and potentially pollination—through presenting pollen with nutrients that meet the visitors' preferences. In contrast, plants that depend little or not on pollinators, e.g., wind- and self-pollinated plants, are unlikely subject to pollinator-mediated selection for pollen nutrients (Baker and Baker 1979). The nutritional content of their pollen may thus differ from pollen of plants that do depend on animals for pollination. Indeed, the large differences often observed for relative amounts of nutrients (e.g., protein content which can range between 2.5 and 61%; Roulston et al. 2000) may be explained by different levels of insect pollinator dependence.

However, from the plant's perspective, the primary function of pollen is reproduction. In this regard, closely related plant species likely require similar amounts of nutrients or nutrient ratios to ensure efficient fertilization due to common metabolic pathways and similar floral morphologies. Although most of the nutrients for fertilization are provided by the mother plant (Labarca and Loewus 1973), pollen needs to carry additional nutrients to remain fertile over the transfer period and to initiate the fertilization process. For example, pollen protein and sugar content play an important role for pollen tube growth (Labarca and Loewus 1973). Similarly, pollen lipids—typically stored in the pollen intine—most likely act as energy storage (Ibrahim 1974). Plants with long styles thus require more protein and energy in the form of sugars or lipids to grow sufficiently long pollen tubes (Roulston et al. 2000). The collection and use of pollen as nutrient source by many flower-visiting animal species (Haydak 1970; Roulston and

Cane 2000; Stanley and Linskens 1974) is thus often of no benefit or even a cost to the plant—particularly if the plant does not rely on animals for pollination, such as wind-pollinated plants—because pollen consumed by animals cannot be used for fertilization. Besides producing overall larger amounts of pollen to increase chances of fertilization (Friedman and Barrett 2009), the exclusion of pollinators could be a reason for the low pollen nutrient amounts typically found in wind-pollinated plants. Differences in pollen nutritional content may, therefore, be largely (or solely) determined by plant species-specific requirements for pollen fertility and thus phylogenetic relatedness, e.g., due to family- or genera-specific metabolic pathways or similar floral morphologies (Hanley et al. 2008; Roulston et al. 2000). Consequently, the nutritional composition of pollen appears to be subject to two different and potentially conflicting selective pressures: the preferences of pollinators and the plant's own fertility which is largely determined by phylogenetic relatedness. These selective pressures may also explain why some animal-pollinated plant species even present two types of stamen: stamen with pollen that specifically serves as reward for pollinators, and stamen with pollen for ovule fertilization (heteranthery: (Vallejo-Marín et al. 2009)).

It is still largely unclear to what extent pollen nutrient content is driven by pollinator needs and/or phylogenetic relatedness. Although pollen did not primarily evolve as reward for pollinators, the plants' dependence on animals for pollination may have altered its nutritional profile over the course of evolution. The few previous studies comparing pollen nutritional content across plant species provided inconsistent results. For example, the ground-breaking work by Roulston et al. (2000) found a phylogenetic signal for pollen protein content, but did not find any influence of the pollination system. By contrast, Hanley et al. (2008) found a significant correlation between pollen protein content and pollination strategy, but did not specifically test for a phylogenetic signal.

Protein was, until recently, considered the main quality feature of pollen for pollinators, especially for bees (DeGroot 1953; Herbert et al. 1977), as it is the most abundant nutrient in pollen (Roulston and Cane 2000) and is considered the most important nutrient for bee larval growth (DeGroot 1953). Recent studies, however, highlight the importance of other pollen nutrients, e.g., lipids, fatty acids (Manning et al. 2007), and sterols (Vanderplanck et al. 2011), as well as the significance of specific ratios between different nutrients (Raubenheimer and Simpson 1999; Vaudo et al. 2016a, b). While a lack of nutrients may simply be compensated by eating more, an unbalanced ratio will automatically lead to over- or under-eating at least some nutrients (Raubenheimer and Simpson 1999; Vaudo et al. 2016a, b). Nutrient amounts differ

strongly between plant species, while very little is known about differences in nutrient ratios between plant species.

Only few studies compared pollen nutritional content across plant species (e.g., Auclair and Jamieson 1948; Baker and Baker 1979; Somerville 2001; Todd and Bretherick 1942; Weiner et al. 2010), and most of these studies focused on either one or few plant species and analyzed only one or a subset of nutrients, while studies analyzing a broader spectrum of plant species and nutritional components remain scarce (e.g. Somerville 2001; Todd and Bretherick 1942). Moreover, many previous studies analyzed bee-collected pollen, which usually contains salivary secretions added by bees, i.e., regurgitated nectar to facilitate pollen handling (Winston 1991), which contain nutrients and may alter the nutrient composition of the analyzed pollen (Roulston and Cane 2000). More importantly, bee-collected pollen by definition reflects bee preferences and may thus not be fully representative for the flowering plant community. Hence, the analysis of hand-collected pollen would provide a more accurate picture of pollen nutrients.

Here, we conducted a meta-analysis on the data sets published in 70 studies (Supplementary Material S1) to better understand which factors (phylogenetic relatedness and/or pollinator attraction) are associated with (1) pollen nutritional content and (2) nutrient ratios. We tested for a phylogenetic signal and an effect of insect dependence (i.e., full, high, low, or no insect pollinator dependence) on various nutrients as well as their ratios. We expected (1) a phylogenetic signal in pollen nutritional content as a consequence of phylogenetic relatedness. Due to the strong effect of pollen nutritional quality on pollinator fitness and thus flower choice behavior, we further hypothesized that insect dependence is correlated with pollen nutrient content and the dietary requirements of their (main) pollinators. If so, we expected (2) this to result in differences in nutritional content between different levels of insect pollinator dependence, which are independent of the plants' phylogenetic relatedness. Notably, there is no common quality parameter that serves all pollinators. Instead, nutritional requirements may depend on different pollinator species/groups. Unfortunately, very little is known on nutritional requirements of different pollinators. As our data set is largely confined to insect-pollinated plants, we primarily took into account nutritional requirements of insect herbivores, many of which were found to regulate protein intake (Simpson and Raubenheimer 2012). As protein and fat further appear to be the most important and most abundant nutrients in pollen and to affect the foraging behavior of bees (Leonhardt and Blüthgen 2012; Roulston and Cane 2000; Vaudo et al. 2016a, b), we hypothesized to find differences in contents and ratios particularly for these two nutrients.

Materials and methods

We compiled data from the literature on the nutritional content of one or more pollen nutrients. In total, our study includes 387 different plant species belonging to 229 plant genera in 75 different families (Fig. 1, Supplementary Material, Table S1). We included all studies, which provided data on sugar content, crude protein content, polypeptide content, free amino acid content and/or lipid content of pollen as well as information about whether the pollen was bee-collected (58.6% of the data set) or hand-collected (41.4%) (Supplementary Material, Table S1). As most of the studies on carbohydrate content solely included sugars, while some others included all carbohydrates or both, we included only the sugar content (but still refer to “protein-to-carbohydrate” ratio, which is the commonly used term in most studies). For the units (w/w or percentages of dry weight) in which nutrient contents were reported varied between studies, we only used studies with clearly defined units and converted all units into percentages of total pollen dry weight. We further calculated the protein-to-carbohydrate (P:C) ratio and the protein-to-lipid (P:L) ratio based on nutrient percentages.

As insects are typically the most abundant and most common pollinators (Faegri and Van der Pijl 2013) and insect pollination is considered the oldest form of pollination (Labandeira and Currano 2013), the majority of animal-pollinated plants in our study was found to be pollinated by insects with only few bird- and mammal-pollinated species [e.g., Australian *Banksia* species (Hopper 1980)]. We thus included only insect-pollinated plants in our analyses, as the degree of dependence on animal pollination is hardly known for other plants.

Where available (~70% of plants in the data set), we assigned the level of insect pollinator dependence to each plant species based on information provided by the BioFlor database v 1.1 (<http://www2.ufz.de/biolflor/index.jsp>, accessed in April 2019). We classified plants as (1) fully dependent on insect pollinators when they need insects for pollination and are self-incompatible ($N=103$, ca. 43% of the data set); (2) highly dependent on insect pollinators when they are mostly insect pollinated, but can self-pollinate and are self-compatible ($N=76$, ca. 32%); (3) little dependent on insect pollinators when they are mainly wind- or self-pollinated, but can also be pollinated by insects ($N=36$, ca. 15%); and (4) independent of insect pollinators when they are exclusively wind- or self-pollinated ($N=24$, ca. 10%). Classifications 3 and 4 were combined in one category (i.e., low to none dependence on insect pollinators) in our statistical analyses.

All analyses were performed at the plant species level. Initial data screening with generalized linear models

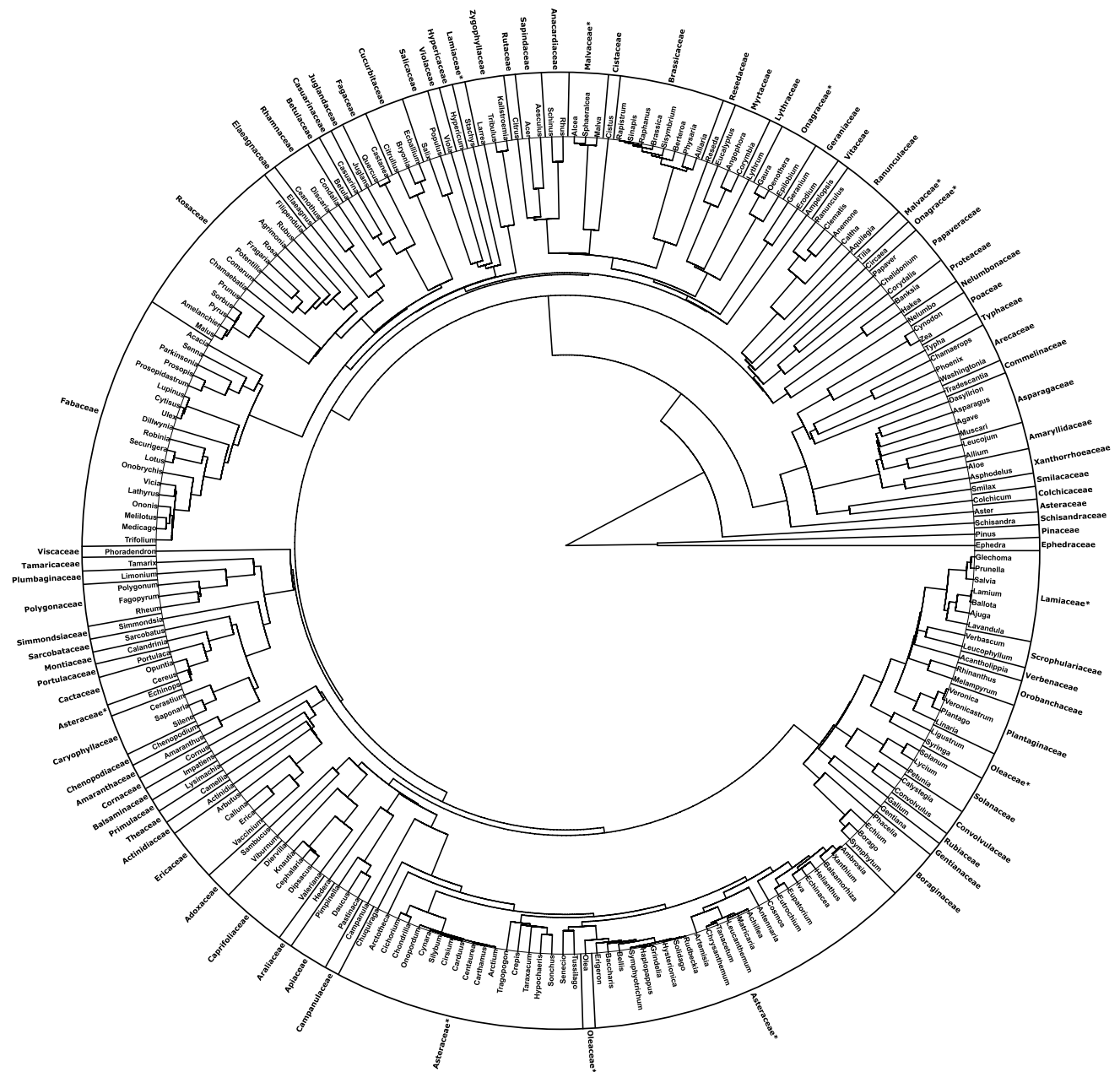


Fig. 1 Phylogenetic tree of the plant genera included in this meta-analysis. Plant families are indicated along the outer circle of the tree. The tree was generated based on the molecular phylogeny of Zanne et al. (2014) using the *pez* package in R. Asterisks behind fam-

ily names indicate families that appear at least twice in the generated phylogeny. Isolated single genera that were not placed in the correct family were excluded from subsequent phylogenetic analyses

(GLMs, McCullagh 2018) revealed significant interactions between nutrient content and collection method (i.e., whether pollen was bee- or hand-collected, Table 1). To assess whether the effect of collection method was due to differences in nutrient contents or to differences in the selection of plant species in each data set, we restricted the data set to those plant species that were found in both original data sets ($N=29$) and performed separate *t* tests to test for differences in nutrient contents between

collection methods. For this data set, we found differences in the sugar content of pollen of the same plant species (Table 1). Interestingly, the mean relative lipid content was also higher in hand-collected than in bee-collected plants. We therefore treated both data sets separately in subsequent analyses.

We then tested for a phylogenetic signal in nutritional content following Junker et al. (2017). We used Blomberg’s *K* to test whether nutrient contents correlated with phylogeny

Table 1 Results of general linear models (GLMs) [F and P ($>F$)] testing for a significant interaction between nutrient content and collection method (for the entire data set), and t tests [t and P ($>t$)] testing for differences in the relative contents (% dry weight) of the three

Nutrients	Mean bee-collected	Mean hand-collected	F	P ($>F$)	t	P ($>t$)
Crude protein ($N=29$)	0.932 \pm 0.28	1.019 \pm 0.46	1.814	0.079	-1.029	0.312
Polypeptides ($N=27$)	1.051 \pm 0.20	1.000 \pm 0.65	2.037	0.161	-1.221	0.285
Free amino acids ($N=21$)	2.296 \pm 1.66	1.639 \pm 0.95	1.484	0.169	0.780	0.449
Lipids ($N=18$)	0.888 \pm 0.89	2.190 \pm 0.86	2.737	0.004	0.282	0.782
Sugars ($N=9$)	1.697 \pm 0.54	0.807 \pm 0.42	5.340	0.033	2.854	0.028

Shown are the standardized means (\pm SD) of each group and t and P values (significance level: $P < 0.05$). Significant P values are marked in bold

and were therefore likely influenced by the evolutionary history of a plant species. Blomberg's K depicts the variance between phylogenetic clades in relation to the variance within clades. The underlying phylogenetic tree was based on the recent molecular phylogeny of Zanne et al. (2014) restricted to those plant genera that were included in our analysis (Fig. 1). The tree was constructed using the R-package *pez* (Pearse et al. 2015). Missing species were bound, and terminal branches pruned to produce a cladogram.

For the bee-collected pollen data set, we subsequently performed phylogenetic analyses of variance (phyl-ANOVA) for each nutrient group and ratio to test for differences between the different pollination dependence levels independent of plant relatedness (using the R-package *phytools*). Post-hoc pairwise comparisons were corrected with false discovery rate (FDR, Benjamini and Hochberg 1995). Unfortunately, the small sample size for the hand-collected data set precluded equivalent statistical tests, and we, therefore, present the data on hand-collected samples only graphically (Figure S1). To further assess whether contents of different nutrients were correlated, e.g., due to linked biochemical pathways, we used phylogenetic generalized least squares (PGLS) models fit by maximum likelihood with Brownian correlation (using the R-package *nlme*). P values were corrected for multiple testing using FDR. All statistical tests were performed in R v3.5.1 (R Core Team 2018).

Results

Pollen crude protein content and the P:L ratio showed a clear phylogenetic signal (Blomberg's K) for both the bee- and hand-collected pollen data set (Table 2). Polypeptides showed a phylogenetic signal only for hand-collected pollen (Table 2). Contents and ratios of all other nutrients were not correlated with plant phylogenetic relatedness (Table 2).

In bee-collected pollen, the crude protein content was further higher in plants that are fully or highly dependent on insect pollinators, independent of their phylogenetic relatedness (Table 3; Fig. 2a). The protein-to-lipids ratio

main macronutrients as well as polypeptides and free amino acids between the two different collection methods (hand- or bee-collected, for a data set reduced to 29 plant species that were included in both collection methods)

Table 2 Results of Blomberg's K test testing for a phylogenetic signal for each nutrient for the bee- and hand-collected data set

Nutrients	Collection method	K	P
Crude protein ($N=179$)	Bee	0.199	0.001
	Hand	0.237	0.016
Polypeptides ($N=167$)	Bee	0.029	0.968
	Hand	0.828	0.001
Free amino acids ($N=79$)	Bee	0.541	0.391
	Hand	0.134	0.509
Lipids ($N=67$)	Bee	0.054	0.538
	Hand	0.416	0.114
Sugars ($N=38$)	Bee	0.165	0.216
	Hand	0.082	0.849
Protein:carbohydrate ratio ($N=38$)	Bee	0.077	0.739
	Hand	0.605	0.101
Protein:lipid ratio ($N=59$)	Bee	0.208	0.012
	Hand	0.776	0.029

Shown are Blomberg's K and P values. Significant P values after Benjamini–Hochberg correction (FDR) are marked in bold

Table 3 Results of phylogenetic ANOVAs testing for differences in the relative contents (% dry weight) and ratios of the four main macronutrients between different levels of insect pollinator dependence for the bee-collected pollen data set

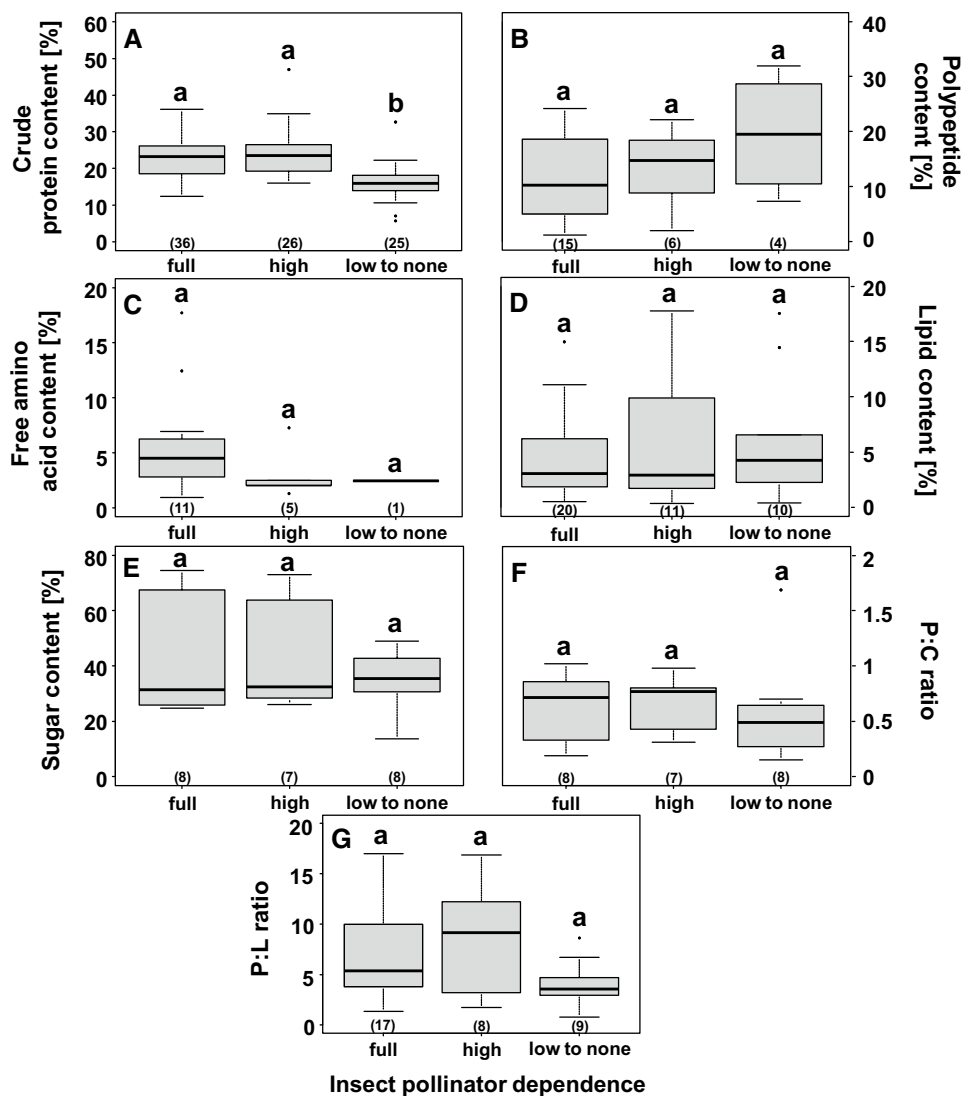
Nutrients	F	P
Crude protein ($N=87$)	14.345	0.001
Polypeptides ($N=25$)	1.3891	0.275
Free amino acids ($N=17$)	0.820	0.314
Lipids ($N=41$)	0.652	0.473
Sugars ($N=23$)	0.664	0.525
Protein:carbohydrate ratio ($N=23$)	0.063	0.940
Protein:lipid ratio ($N=34$)	2.094	0.095

Shown are F and P values

The number of plant species (N) for each nutrient is indicated in brackets behind each nutrient

P values indicating significant differences after Benjamini–Hochberg correction (FDR) between pollination strategies are marked in bold

Fig. 2 Differences in the relative content (% pollen dry weight) of crude protein (a), polypeptides (b), free amino acids (c), lipid (d) and sugars (e), as well as the protein-to-carbohydrate ratio (P:C ratio, f) and protein-to-lipid ratio (P:L ratio, g) of plants differing in the degree of insect pollinator dependence for bee-collected pollen. Numbers in brackets below boxplots give the numbers of plant species included in each group. Different letters above boxes indicate significant differences between different degrees of insect pollinator dependence (following pairwise comparisons of the phylogenetic ANOVA post-hoc tests corrected for multiple testing). Boxplots represent the median (central mark), the 25th and 75th percentiles (edges of the boxes), the most extreme data points (whiskers). Outliers (outside of the range of $1.5 \times \text{IQR}$) are plotted individually (dots)



also tended to be higher in plants that are fully or highly dependent on insect pollinators, independent of their phylogenetic relatedness (Table 3; Fig. 2g). Free amino acids, polypeptides, and lipids, the protein-to-carbohydrate ratio as well as pollen sugar content (Table 3; Fig. 2c, e) did not significantly differ between different levels of insect pollinator dependence.

For bee-collected pollen, contents of crude protein, free amino acids, and polypeptides were correlated (Table 4), and the relative amount of crude protein was correlated with the relative amounts of lipids and sugars (Table 4). In addition, the relative amount of lipids was correlated with the relative amount of polypeptides (Table 4). For the hand-collected data set, only the relative amount of crude protein was correlated with the relative amount of free amino acids and polypeptides (Table 5).

Discussion

In this meta-analysis, we show that the degree of a plant species' dependence on insects for pollination correlated with differences in the crude protein content of pollen, independent of the plants' phylogeny. In line with our hypothesis of pollinator-mediated selection, pollen protein content increased with increasing dependence on insect pollinators (Fig. 2a). Our study also revealed a significant phylogenetic signal for crude protein and the P:L ratio of pollen. Variation in the relative content of other nutrients did not clearly correlate with phylogenetic relatedness, indicating that phylogenetic relatedness alone may not always explain variation in overall pollen nutritional content, as has recently also been shown for pollen-kitt lipids

Table 4 Phylogenetic generalized least squares (PGLS) models fit by maximum likelihood with Brownian correlation denoting relationships between the four main macronutrients and free amino acids in bee-collected pollen

Nutrients	Polypeptides	Free amino acids	Lipids	Sugar
Crude protein	$t = 3.185$ $P = 0.002$ ($N = 25$)	$t = 3.434$ $P < 0.001$ ($N = 20$)	$t = 5.712$ $P < 0.001$ ($N = 76$)	$t = 2.888$ $P = 0.007$ ($N = 29$)
Polypeptides	–	$t = -16.245$ $P < 0.001$ ($N = 23$)	$t = 4.033$ $P = 0.002$ ($N = 14$)	$t = -1.007$ $P = 0.335$ ($N = 13$)
Free amino acids	–	–	$t = -0.217$ $P = 0.833$ ($N = 13$)	$t = 1.813$ $P = 0.103$ ($N = 11$)
Lipids	–	–	–	$t = -0.815$ $P = 0.421$ ($N = 33$)

Given are t and P values. Significant P values after Benjamini–Hochberg correction (FDR) are marked in bold

N gives the number of plant species included in each correlation analysis

Table 5 Phylogenetic generalized least squares (PGLS) models fit by maximum likelihood with Brownian correlation denoting relationships between the four main macronutrients and free amino acids for the hand-collected pollen

Nutrients	Polypeptides	Free amino acids	Lipids	Sugar
Crude protein	$t = 3.402$ $P = 0.006$ ($N = 13$)	$t = 3.272$ $P = 0.010$ ($N = 11$)	$t = 1.008$ $P = 0.328$ ($N = 18$)	$t = -0.312$ $P = 0.760$ ($N = 17$)
Polypeptides	–	$t = 0.605$ $P = 0.546$ ($N = 133$)	$t = 0.536$ $P = 0.620$ ($N = 6$)	$t = -0.682$ $P = 0.544$ ($N = 5$)
Free amino acids	–	–	$t = -0.822$ $P = 0.497$ ($N = 4$)	$t = -2.348$ $P = 0.143$ ($N = 4$)
Lipids	–	–	–	$t = 0.085$ $P = 0.933$ ($N = 15$)

Given are t and P values. Significant P values after Benjamini–Hochberg correction (FDR) are marked in bold

N gives the number of plant species included in each correlation analysis

(Chichiricò et al. 2019). Notably, detecting phylogenetic signals with the help of statistical tools is a useful measure of pattern, but cannot be interpreted as evidence of acting evolutionary processes (Revell et al. 2008).

Alternatively, pollen nutritional variation may be explained by biotic or abiotic factors, such as pollinator requirements, soil quality (Lau and Stephenson 1993), surrounding plant species (Sargent et al. 2011), temperature (van der Kooi et al. 2019a) as well as plant species-specific traits. For example, an increased style length may require the pollen grain to contain higher amounts of nutrients involved in pollen tube growth, which might result in significant differences in macro-nutrient (i.e., protein, fat, and carbohydrate) content.

Our finding that pollen protein content was considerably higher in plants that fully or highly depend on insect pollinators compared to plants with low insect dependence, independent of phylogenetic relatedness, suggests that the importance of pollen protein could exert a considerable

selective pressure on animal-pollinated plants. Indeed, bumblebees prefer plants with pollen of comparatively high protein content or a high P:L ratio (Leonhardt and Blüthgen 2012; Ruedenauer et al. 2016; Somme et al. 2015; Vaudo et al. 2016b), which may be due to the importance of pollen nutritional quality for insect larval development (Haydak 1970; Herbert et al. 1977; Raubenheimer and Simpson 1999). As a consequence, plants that highly depend on animals for pollination may produce pollen of a comparatively higher protein content compared to plants with low or no dependence on insect pollinators (Fig. 2a). Plants that do not or weakly rely on animals for pollination clearly also produce some pollen nutrients, because those nutrients are necessary for pollen germination and/or pollen tube growth. Pollinators are known to collect pollen also from these plants (Saunders 2018) and likely transfer pollen in the process, which seemingly contradicts the apparent preference of pollinators for pollen of high protein content. However, this behavior may be explained

by the pollinators' need to mix pollen of different plant species to, e.g., dilute toxic pollen or adjust nutrient ratios (Simpson and Raubenheimer 2012) or because these plants may, at times, simply be the only nutrient sources available (Ackerman 2000).

For both the bee- and hand-collected data set, we further found crude protein, free amino acids, and polypeptides to be correlated, which may be due to shared biochemical pathways (Külheim et al. 2009). In addition, lipids were correlated with crude protein, sugar, and polypeptides at least in the bee-collected data set. The ratio of protein to lipids was found to strongly affect bumblebee foraging preferences with lipid intake being more strongly regulated than protein intake (Vaudo et al. 2016b). From the bees' perspective, a correlation between these two nutrient groups would enable them to more easily assess and potentially even regulate both macronutrients and their ratio simultaneously, e.g., by mixing pollen from different plants (Kriesell et al. 2017). After all, pollen is a complex mixture of a relatively high number of different substances, potentially rendering it challenging for pollinators to assess them all simultaneously. Moreover, amino acids correlate with each other (Weiner et al. 2010) as well as with the total protein content across plants. Bees do, therefore, not need to perceive all amino acids, because it suffices to perceive some amino acids to make inferences on overall amino acid/protein content (Ruedenauer et al. 2019).

Interestingly, earlier studies on pollen protein content revealed partly contrasting results. While Roulston et al. (2000) also found a phylogenetic signal for crude pollen protein, they did not find differences between different pollination strategies as found in our analysis and by Hanley et al. (2008). This discrepancy could be due to different statistical approaches. In addition, a potential limitation of meta-analyses on pollen nutrients is that extraction protocols and analytical methods applied usually differ among studies, which increases overall variance. For example, in our data set, highly different analytical approaches were used to analyze pollen lipid content, some being highly lipid specific, others also extracting additional non-polar substances besides lipids. We could, unfortunately, not restrict our data set to studies which performed more specific analyses due to the overall small sample size of studies which have analyzed pollen lipids. Future studies using standardized analytical methods for different nutrients and high numbers of plant species should provide more robust data sets.

In conclusion, our results suggest that variation in pollen nutritional content is not only determined by phylogenetic relatedness, but also other factors, likely including selection imposed by pollinator preferences or by plant species' traits demanding high pollen nutrient contents. In plant species that are fully or highly dependent on insect pollinators, pollen nutrient contents and ratios might have co-evolved with the needs of their insect pollinator partners.

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Author contribution statement SDL, JS, and FAR conceived the study. The data set was compiled and edited by FAR. Statistics were designed and performed by SDL and FAR. FAR, SDL, CJvdK, and JS wrote the manuscript. All authors discussed the results, commented on the paper, and agreed to the final version.

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Data availability Data available from https://osf.io/2j86m/?view_only=a37e8a80e7e64f16b0c93323a238f1d8.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study formal consent is not required.

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