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Differential effects of pollen nutritional quality on male and female reproductive success within a diverse co-flowering community

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Abstract

1. Pollen protein content has been demonstrated to be an essential nutritional component for bees and thus important in mediating plant–pollinator interactions. However, little is known on the drivers and consequences of among-species variation in pollen protein content and how this can impact male and female reproductive success across plant species. Among-species variation in resources allocated to pollen nutrition could further be constrained by life-history strategies (e.g. survival-reproduction trade-offs) or evolutionary history.
2. Here, we surveyed pollen protein content for 29 species within a diverse co-flowering community and evaluated the effect of pollen protein on male and female reproductive success. We also tested the role of life history (annuals vs. perennials) and phylogeny in mediating differences in resource allocation to pollen nutrition.
3. We found that pollen protein content influences components of male (bee visitor abundance and pollen dispersal) but not female (conspecific pollen deposition and pollen tube growth) reproductive success, suggesting this trait affects plants only via male function. This sex-specific effect further suggests the potential for sexual conflicts driven by differential investment on this trait. We found no phylogenetic signal on pollen protein content. However, pollen protein content was higher in annual compared to perennial species suggesting survival versus reproduction trade-offs also contribute to variation in pollen protein at the community level.
4. Our study underscores the importance of understanding the ecological and evolutionary drivers of pollen protein content across plant species. Our results further suggest the existence of sexual conflicts and ecological trade-offs mediated by differential investment in pollen nutritional quality, with important implications for community assembly and the structure of plant–pollinator interactions.

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KEYWORDS
annual plants, nutritional ecology, plant–pollinator interactions, pollen dispersal, pollen protein, sexual conflicts

1 | INTRODUCTION

Plants vary widely in the macronutrients (e.g. lipids and proteins) contained in their pollen (Vaudo et al., 2024), with over a three-fold difference across surveyed plant species (Vaudo et al., 2020, 2024; Yeamans et al., 2014). However, the ecological drivers and consequences of such variation within co-flowering communities remain largely unknown (but see Vaudo et al., 2024). Moreover, while the benefits of pollen macronutrients for pollinator fitness, particularly that of pollinivorous pollinators (e.g. bees), have been well studied (Cardoza et al., 2012; Chau & Rehan, 2024; Crone & Grozinger, 2021; Di Pasquale et al., 2013; Radev, 2019; Roulston & Cane, 2002; Stephen et al., 2024), the relationship between pollen macronutrients and plant reproductive success is much less understood. Given that pollen nutritional quality is essential for pollinivorous pollinators (Crone & Grozinger, 2021; Roulston & Cane, 2002), plant investment in this trait may be shaped by selection to maximize plant fitness via its role in pollinator foraging behaviour. For instance, pollen protein content has been shown to be positively associated with bee body size (Roulston & Cane, 2002), stress tolerance (Crone & Grozinger, 2021; Di Pasquale et al., 2013) and reproductive output (Cardoza et al., 2012; Radev, 2019; Ruedenauer et al., 2019). It has also been shown that bees can discriminate between pollen sources with different pollen protein levels across and within plant species (Cardoza et al., 2012; Leonhardt & Blüthgen, 2012; Russo et al., 2019, 2023; Vaudo et al., 2016; Yeamans et al., 2014). It is thus possible to expect that among-species differences in pollen protein levels may differentially impact plant reproductive success by influencing bee visitation and foraging behaviour. However, how investment in pollen protein content relates to plant reproductive success across multiple species within a co-flowering community is not known. Such knowledge would aid in our understanding of the role of pollen protein content in mediating plant–pollinator interactions, pollinator-mediated competition and coexistence within plant communities.

An increase in overall plant fitness can be achieved via an increase in male and/or female reproductive success (Bergamo et al., 2024; Charnov, 1979; Muchhala & Thomson, 2012; Sutherland & Delph, 1984; Willson, 1979), both of which could be influenced by pollen protein content. Male reproductive success could increase if higher pollen protein levels increase the probability of pollen dispersal to conspecific flowers (Muchhala et al., 2010; Muchhala & Thomson, 2012; Ruedenauer et al., 2019; Stanton et al., 1991). This may be achieved by increasing the total number of visits to flowers, the diversity of visitors or the total number of pollen grains dispersed by individual bees, all of which may contribute to increases in siring success (Muchhala et al., 2010). It has also been proposed that male reproductive success would be highest when pollinators are specialized and visit frequently (Moreira-Hernández & Muchhala, 2019; Muchhala et al., 2010). In this sense, an increase in pollen protein content may lead to higher bee visitation rates and to a higher degree of specialization (Muchhala et al., 2010), hence increasing pollen dispersal to conspecific flowers. On the other hand, female reproductive success could be maximized if an increase in pollen protein content results in higher pollen tube production, ovule fertilization and seed set (Bergamo et al., 2024; Sutherland & Delph, 1984). Studies have shown that pollen protein content can be positively associated with pollen viability (Yeamans et al., 2014) and hence ovule fertilization. Interestingly, it has also been shown that ecological processes (e.g. competition) that take place within co-flowering communities can differentially affect components of male and female success (Bergamo et al., 2024; Ellis & Johnson, 2010), potentially leading to divergent ecological and evolutionary outcomes (Charnov, 1979; Moore & Pannell, 2011; Sutherland & Delph, 1984; Willson, 1979). However, whether pollen protein content differentially affects male and female reproductive success within diverse co-flowering communities has not been studied.

Pollen protein levels may also vary because of trade-offs (survival vs. reproduction) associated with different plant life-history strategies. It is well-known that annual plants preferentially invest in reproduction (e.g. early flowering, large flower number and high-quality rewards) over survival (e.g. defence), while perennials show the opposite pattern (Friedman, 2020). The differential allocation of resources in survival versus reproduction between annuals and perennials has been shown to have important implications for the evolution of sexual and mating systems in plants (Barrett & Harder, 2017; Friedman, 2020; Stebbins, 1950). For instance, self-pollination rates have been shown to be significantly higher in annuals compared to perennial plants (reviewed in Friedman, 2020). Interestingly, annual plants have also been shown to produce larger pollen volumes (Hicks et al., 2016), and to be less pollen-limited than perennials (Knight et al., 2005), supporting their greater allocation toward reproduction (Hicks et al., 2016). Higher pollen protein content, along with higher pollen volumes, may further aid in reducing pollen limitation in annuals via increases in pollinator visitation, thus contributing to the low pollen limitation observed in annual plants at global scales (Knight et al., 2005). Differences in plant life-history strategies, and their associated trade-offs, may contribute to the pollen nutritional landscape observed within highly diverse co-flowering communities (Vaudo et al., 2024), but this remains largely unexplored.

Finally, variation in protein levels across species could also be the result of evolutionary history (Roulston et al., 2000; Ruedenauer et al., 2019). In this case, closely related species would be expected to have similar levels of pollen macronutrients compared to distantly related plants (Roulston et al., 2000; Ruedenauer et al., 2019; Vaudo et al., 2024). Indeed, some research has indicated the existence of consistent patterns within plant families in pollen macronutrient ratios (Vaudo et al., 2024). Yet, others have shown a high degree of plasticity in this trait (Yeamans et al., 2014), with higher levels of variation within plant genera compared to families (Vaudo et al., 2024). Overall, there are few studies that have simultaneously evaluated the importance of the ecological and evolutionary drivers underlying variation in pollen macronutrient levels within a single co-flowering community. Evaluating drivers of variation in pollen protein content across plant species within diverse co-flowering communities is central to uncover its ecological and evolutionary consequences (Vaudo et al., 2024). Selective pressures on pollen protein content, for instance as a result of pollinator competition, may only take place when insects can select among co-flowering plants with varying degrees of pollen protein. Such studies are thus critical to fully understand the potential role of pollen protein levels in mediating plant–pollinator interactions and plant community assembly across spatial and temporal scales. In this study, we have the following objectives: (1) describe the pollen protein landscape in a diverse co-flowering community, (2) evaluate the effects of pollen protein content on components of male and female plant reproductive success, (3) evaluate whether annual and perennial species differ in pollen protein content and (4) assess the role of evolutionary history (phylogenetic signal) underlying among-species variation in pollen protein content.

2 | METHODS

2.1 | Study system

The study was conducted in a diverse co-flowering community at the serpentine seeps in the McLaughlin Natural Reserve (38.8582° N, 122.4093° W) in Northern California, USA, which is part of the University of California Natural Reserve System (Arceo-Gómez et al., 2018; Arceo-Gómez et al., 2016; Arceo-Gómez & Ashman, 2014a, 2014b). Our study system was composed of two seeps (RHA and BS) located less than 1-km apart and with strong similarities in terms of species abundance, diversity and composition (also see Wei, Kaczorowski, et al., 2021). The plant species in this system are adapted to serpentine soils and are restricted spatially and temporally by water availability, limiting the total duration of the flowering season (Freestone & Inouye, 2006). Bees are the main floral visitors in this community including species within Andrenidae, Apidae, Halictidae and Megachilidae (Carneiro et al., 2024; Wei, Russell, et al., 2021). Previous studies have shown that patterns of pollinator visitation, pollen transport and deposition on stigmas as well as plant reproductive success can vary widely among species at these serpentine seeps (Alonso et al., 2013; Arceo-Gómez et al., 2016, 2014a, 2014b).

2.2 | Pollen protein content

Here, we used pollen protein to estimate pollen nutritional quality as it has been shown to play an important role for bee health and reproduction, as well as an important mediator of floral choice (Crone & Grozinger, 2021; Roulston & Cane, 2002; Russo et al., 2023; Vaudo et al., 2020). Pollen protein has also been shown to be one of the main macronutrients closely regulated by bees (Pirk et al., 2010; Ruedenauer et al., 2019). Thus, we assessed the pollen protein content for plant species in bloom at the study site between 15 and 30 May 2023. For this, we collected pollen from at least 10 different flowering plants per species in the field. Using forceps, we collected pollen from dehisced anthers directly into Eppendorf tubes. As the flowers of eight species produced anthers that were too small, too sticky, or not obviously dehiscing, we collected whole anthers. These samples were stored in a freezer and later processed in the laboratory. The samples that consisted of whole anthers were dried in a drying oven (Quincy) at 36°C for 24 h and sifted through a pollen sieve to remove excess plant tissue. No differences in pollen protein content were observed between species for which whole anthers (181.72 ± 96.33 μg/mg) or just pollen was collected (174.81 ± 97.84; $F = 0.02$, $p = 0.86$). We were able to obtain sufficient material for a protein assay for 29 species (~60% of plant community) across 17 plant families (Figures 1 and 2; Table S1).

To quantify protein levels in pollen we used the Bradford Protein assay following a modified protocol from Vaudo et al. (2016). Collected pollen was weighed and placed in a drying oven (Quincy) for 24 h at 36°C. After drying, 1.5 mL of 0.1 M NaOH (Fluka) was added to each pollen sample. To fracture the pollen grains, a Microson Ultrasonicator (Misonix Incorporated) probe was submerged into the solution for 90 sec and samples were subsequently stored for 24 h at 5°C. Immediately prior to testing, the pollen solutions were centrifuged at 2000×*g* for 30 s. The Bio-Rad Protein Assay Kit (Bio-Rad Laboratories) with a bovine γ-globulin protein standard was then prepared following the manufacturer's protocol. The processed samples and the standards were prepared in triplicate and pipetted into a sterile 95-well plate (VWR Avantor). Absorbance readings were taken at 595 nm on a SynergryHi microplate reader using Gen 5.0 software (Biotek). A 5-point calibration curve ($R^2 > 0.97$) was generated to calculate protein levels.

2.3 | Male reproductive success: Pollen dispersal by flower-visiting bees

We used the abundance and richness of bee flower visitors and their pollen dispersal (number of pollen grains carried by individual bees) as components of male reproductive success. We focus on bees as they rely strongly on pollen consumption, particularly compared to other floral visitors such as butterflies and moths (Ollerton, 2017) and thus are expected to be more sensitive to differences in pollen protein. For this, we surveyed the flower-visiting bees for each plant species in the serpentine seep meta-community between 09:00 and

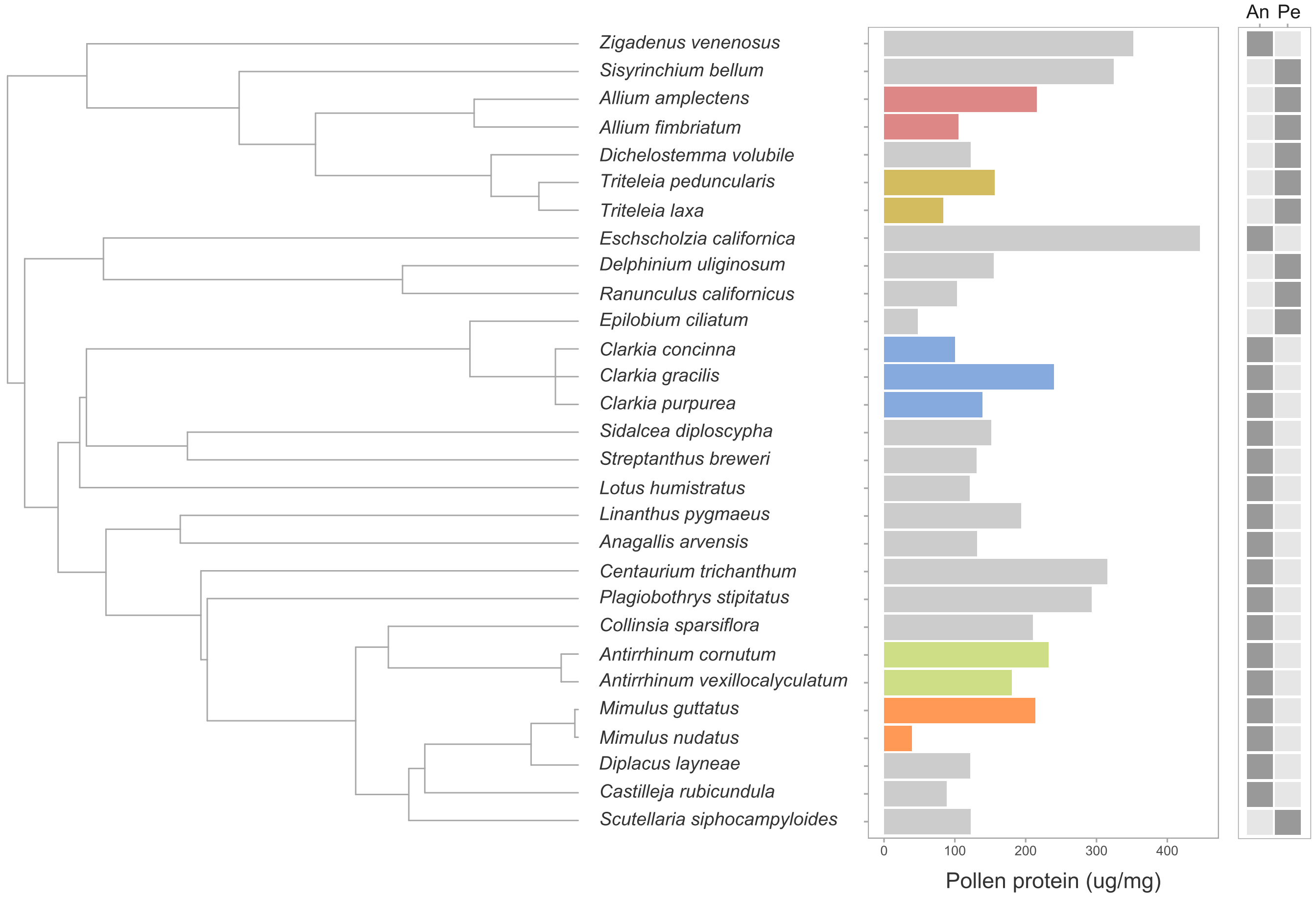

FIGURE 1 Phylogeny of the 29 studied plant species within the co-flowering community in the serpentine seeps, Northern California, USA. Pollen protein content (ug/mg) for each plant species is mapped across the phylogeny (bar graph). Coloured bars (non-grey) indicate species within the same genus. Dark and light grey squares to the right indicate if species are considered annual (An) or perennial (Pe) species. Updated taxonomic names are given in Table S1.

15:00 h using entomological nets over 15 total days between May 9th and June 1st, 2021. This sampling period comprised the flowering season of ~80% of all plant species at the studied community (Wei, Kaczorowski, et al., 2021). Flower-visiting bees were collected by 2–3 people simultaneously walking at a steady pace while observing all plant species and collecting all bees that contacted flower reproductive structures (>120 h of total observation). After direct collection on flowers, the plant host species was recorded, and bee specimens were stored in individual tubes under freezing temperatures to prevent movement and potential pollen loss. In total we collected 621 individual bees, of which 123 were males and 498 females. All specimens were identified at the morphospecies level and are preserved in the pollinator collection at East Tennessee State University. Bee visitor abundance was estimated as the total number of bees collected on a specific plant species. The amount and diversity of pollen dispersed was quantified by sampling the pollen load from bee bodies using a fuchsin jelly cube that was later mounted on a microscope slide (Beattie, 1971). All pollen grains found in bee pollen loads were counted under a microscope and identified based on pollen libraries previously established from each plant species at the study site (Carneiro et al., 2024). Mean pollen dispersal was calculated as the mean number of pollen grains belonging to a specific plant species found in individual bee pollen loads. Pollen disperser richness was estimated as the number of bee morphospecies carrying pollen from a particular plant species.

2.4 | Female reproductive success: Conspecific pollen deposition and pollen tube growth

We obtained data on conspecific pollen deposition on stigmas (i.e. pollen belonging to the recipient species) and number of pollen tubes produced (i.e. pollen tubes that reach the base of the style) for 24 plant species. In total, we collected data from 1279 styles with a mean of 55.6 (±37.1) styles per species. Styles were collected in the field over the span of 3 weeks (each style from a different individual) between May 9th and June 1st, 2021. Only flowers that were at the end of their lifetime (wilted flowers) and thus were no longer available for pollination, were used for style collection. Styles were stored in 70% ethanol until processing and softened and stained with aniline blue using standard methods in the laboratory (Arceo-Gómez et al., 2016; Dafni, 1992). Pollen loads on the stigma were

FIGURE 2 Representative examples of the plant species in this study. Photos of flowers and pollen grains (scale bars at 20 μm) for 14 species (approximately 50% of the data set) are shown. All species grow at the serpentine seep communities at McLaughlin Natural Reserve, Northern California, USA. The species are: (a) *Allium amplectens* Torr. (Amaryllidaceae). (b) *Anagallis arvensis* L. (Primulaceae). (c) *Antirrhinum cornutum* Benth. (Plantaginaceae) visited by *Osmia* sp. (Megachilidae). (d) *Castilleja rubicundula* (Jepson) Chuang & Heckard (Orobanchaceae). (e) *Clarkia concinna* (Fisch. & C. A. Mey.) Greene (Onagraceae). (f) *Delphinium uliginosum* Curran (Ranunculaceae). (g) *Eschscholzia californica* Cham. (Papaveraceae) visited by *Bombus vosnesenskii* Radoszkowski, 1862 (Apidae). (h) *Mimulus guttatus* (Phyrmaceae). (i) *Diplacus layneae* (Greene) G. L. Nesom visited by *Calliopsis* sp. (Andrenidae). (j) *Triteleia laxa* Benth. (Asparagaceae) visited by a male bee. (k) *Scutellaria siphocampyloides* Vatke (Lamiaceae). (l) *Sidalcea diploscypha* (Torr. & A. Gray) A. Gray (Malvaceae) visited by a male bee. (m) *Streptanthus breweri* A. Gray (Brassicaceae) visited by a Megachilidae species. (n) *Zigadenus venenosus* (S. Watson) Rydb. (Melanthiaceae) visited by *Andrena astragali* Viereck and Cockerell, 1914 (Andrenidae).

visualized using a compound microscope at 400× magnification. We recorded the total number of conspecific pollen grains and counted the number of pollen tubes that reached the base of the style under a fluorescent microscope (Arceo-Gómez & Ashman, 2011, 2014a, 2014b). The number of pollen tubes has been typically shown to be a good proxy of pollination success (Alonso et al., 2013; Arceo-Gómez & Ashman, 2014a, 2014b). For each plant species, we estimated average conspecific pollen deposition and the average proportion of pollen tubes (total pollen tubes/total conspecific pollen grains on stigma) that reached the base of the style.

2.5 | Flower abundance

The number of open flowers produced (flower abundance) can also affect the number and diversity of visitors as well as the number of pollen grains dispersed and deposited on stigmas. Thus, to evaluate the potential influence of flower abundance on our estimates of male and female reproductive success, we estimated the total number of open flowers available for each plant species during the same period of bee and style collection. For this, we set 40 plots (1×2-meter each) along the serpentine seeps and recorded the number of open flowers per species every 2 days within a three-week time period. We estimated flower abundance as the total number of open flowers in all plots during the three-week period for each species.

2.5.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Species	Species	29 species
Species	Annuals versus perennials	10 annual, 19 perennial species

2.6 | Data analysis

We used generalized linear models (function *glm*, package 'lme4'; Bates et al., 2015) to test for the relationship between pollen protein content and the following components of male reproductive success across plant species: (1) total bee abundance, estimated as the total number of visiting bees collected on a given species, (2) pollen disperser richness (bee richness), estimated as the number of bee morphospecies carrying pollen from a given plant species and (3) the average number of pollen grains from a plant species carried per individual bee (mean pollen dispersal). All these variables are predicted to impact pollen dispersal and hence siring success. We also evaluated the relationship between pollen protein content and two components of female reproductive success: (1) the average number of conspecific pollen grains deposited on stigmas and (2) the mean proportion of pollen tubes (pollen tubes/pollen grains) that reached the base of the style. These variables have been shown to relate with seed production (Alonso et al., 2013; Arceo-Gómez & Ashman, 2014a, 2014b). We ran a separate linear model for each component of male and female reproductive success with average plant species pollen protein content (μg/mg) as a predictor variable. For all models, we used a Shapiro–Wilk test (function *shapiro.test*) to determine whether the residuals were normally distributed. Where the residuals failed the Shapiro–Wilk test, we log-transformed the response variable. We also ran generalized linear models, using the same procedure as above, to directly test for the potential effect of flower abundance (predictor variable) on each of our components of male and female reproductive success. Flower abundance (count data) was log-transformed.

To evaluate whether pollen protein content differed between annual and perennial plant species we categorized each species according to information available in the literature and our own knowledge of the plants at the study sites. In total we had 10 annual and 19 perennial plant species (Figure 1; Table S1). We used a GLM with Gaussian error-distribution, including pollen protein content as the response variable and life habit (annual vs. perennial) as a predictor.

Finally, to test for the role of evolutionary history in determining plant pollen protein content levels, we tested for phylogenetic signal on this variable. To do this, we built a phylogenetic tree of the plant species in our study using *phylo.maker* in the package "V.PhyloMaker" (Jin & Qian, 2019). We then tested for phylogenetic signal in pollen protein content across species using the function *phylosignal* (package 'picante'; Kembel et al., 2010), which calculates a K statistic of phylogenetic signal as well as a *p*-value based on 999 tip shuffling randomizations (Kembel et al., 2010). All statistical analyses were conducted in R version 4.2.2 (2022).

3 | RESULTS

3.1 | Variation in pollen protein content

Pollen protein content varied widely across all 29 plant species in the serpentine seep community, ranging from 39 μg/mg (*Mimulus nudatus*) to 446 μg/mg (*Eschscholzia californica*), with a mean of 176.7 ± 95.7 μg/mg (Figure 1). We found no evidence of a phylogenetic signal on the observed variation in pollen protein content ($K = 0.10$, $p = 0.710$, Figure 1). However, life-history strategy significantly explained variation in pollen protein content (Figures 1 and 2). Specifically, we observed a significant difference in pollen protein content between annual and perennial plant species ($F = 4.90$, $df = 1$, $p = 0.03$) (Figure 2). The average pollen protein content was higher in annual (203.5 ± 104.8 μg/mg) compared to perennial species (125.9 ± 46.45 ug/mg; Figure 2).

3.2 | Flower visitor abundance, richness and pollen dispersal

We collected a total of 621 bees (78 morphospecies) visiting the studied plants in the community. Bee abundance per plant species ranged from one (*Allium amplectens* and *Castilleja rubicundula*) to 97 (*Clarkia gracilis*), with a mean of 29.6 (±30.9, median = 18) individual bees collected on a plant species. The average number of pollen grains of each plant species carried by bees (pollen dispersal per plant species) was 131.4 (±204.3) across all species in the community, ranging from one (*Lotus humistratus*) to 1041 (*Plagiobothrys stipitatus*) pollen grains carried per bee. The total number of bee morphospecies dispersing pollen for each plant species (pollen disperser richness) ranged from one (*Dichelostemma volubile* and *L. humistratus*) to 49 (*Antirrhinum cornutum*), with a mean of 20.2 (±13.5, median = 20; Figures 3 and 4).

3.3 | Influence of pollen protein content on male and female plant fitness

Components of male reproductive success varied positively as a function of pollen protein content across plant species in the community (Table 1). Bee visitor abundance and the mean number of pollen grains dispersed by bees significantly increased with increasing pollen protein content across plant species (Table 1; Figure 2a,b). However, we did not find a significant relationship between pollen protein content and pollen disperser richness (Table 1, Figure S1), which was only influenced by plant species flower abundance (Table 2). Flower abundance also influenced bee visitor abundance but not the mean number of pollen grains dispersed (Table 2, Figure S2). We observed, on average, 68.6 (±86.2, median = 36.5) conspecific pollen grains deposited on stigmas across species, ranging from zero (*Scutellaria siphocampyloides*) to 413.7 (*C. gracilis*). On average, 18.3% of conspecific pollen grains deposited successfully grew pollen tubes that reached the base of the style across species, with a maximum of 74.6% (*Collinsia sparsiflora*). However, neither pollen protein content nor flower abundance affected any component of female reproductive success, that is conspecific pollen deposition and proportion of pollen tubes (pollen tube: conspecific pollen ratio; Tables 1 and 2, Figures S1 and S2).

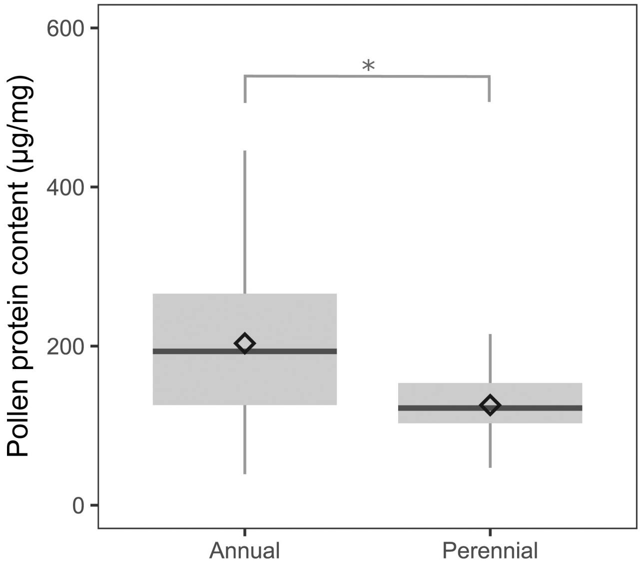

FIGURE 3 Difference in pollen protein content between annual ($n = 10$) and perennial ($n = 19$) plant species growing within a co-flowering community in the serpentine seeps, Northern California, USA. Box plot shows the mean (diamond), median (black line), first and third quartiles (grey box). Asterisk denotes significant differences at $p < 0.05$.

4 | DISCUSSION

Pollen nutritional quality can play a key role in mediating the interactions between plants and bee pollinators, nonetheless its importance, and how it varies across spatial and biological scales, is seldom considered when evaluating the drivers and consequences of these interactions (but see Parreño et al., 2022; Vaudo et al., 2024). Here we show a wide range of pollen protein content across 29 co-flowering plant species in a serpentine seep community. Surprisingly, little is known about the potential ecological and evolutionary implications of such wide variation across plant species (but see Vaudo et al., 2024), although the role of pollen protein content on bee ecology and health has been well-studied (e.g. Chau & Rehan, 2024; Di Pasquale et al., 2013; Pirk et al., 2010; Stephen et al., 2024). Our results further highlight the ecological importance of pollen protein content in plants by demonstrating its indirect effects on plant reproductive success. Specifically, we found a positive relationship between pollen protein content and two components of male reproductive success in plants. Both the total number of visiting bees and the number of pollen grains dispersed on bees were positively affected by pollen protein content. Although bee visitor abundance was also influenced by flower abundance, the average number of pollen grains dispersed was influenced by pollen nutritional quality alone. Previous studies have shown that the intake of pollen protein can be regulated by bees (Pirk et al., 2010; Vaudo et al., 2016). It has also been shown that bees can differentiate between plants providing different pollen quality and show preferences for plants offering pollen with high protein content (Russo et al., 2019, 2023). Thus, higher pollen protein content likely leads to increases in bee visitation rates and overall pollen dispersal (number of pollen grains carried per bee), which has been associated with increases in siring success in plants (Mitchell et al., 2009; Moreira-Hernández &

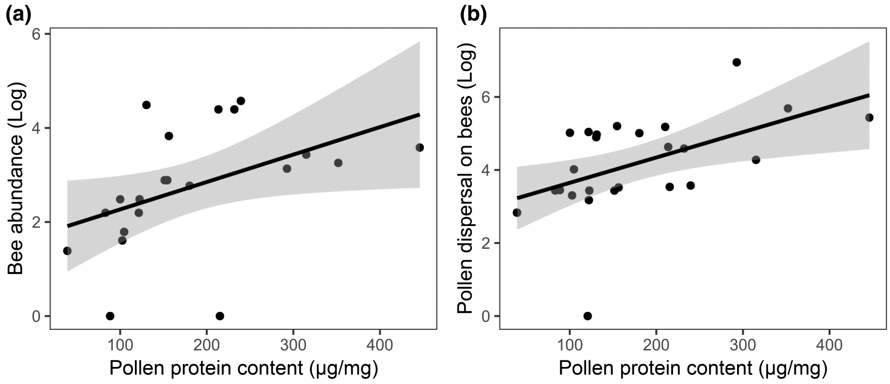

FIGURE 4 Relationship between pollen protein content and (a) visitor abundance (total number of insects collected on each plant species) and (b) the average number of pollen grains per plant species dispersed by insects. Response variables were log-transformed. Both relationships were statically significant ($p < 0.05$), the grey area around the lines indicates 95% confidence intervals.

TABLE 1 Results from generalized linear models and likelihood-ratio tests to evaluate the effects of pollen protein content on pollination components associated with male (pollen dispersal) and female (ovule fertilization) fitness.

	Fitness component	Effect size	*F*-value	*p*-value
Male fitness	**Bee abundance (Log)**	**0.006**	**4.85**	**0.040**
	Pollen dispersal on bees (Log)	**0.007**	**7.66**	**0.012**
	Pollen disperser richness (bee richness)	0.045	2.61	0.120
Female fitness	Mean conspecific pollen deposition (Log)	0.001	0.11	0.740
	Pollen tube:Conspecific pollen ratio (Log)	0.001	0.16	0.690

Note: Significant contrasts are shown in bold.

TABLE 2 Results from generalized linear models and likelihood-ratio tests to evaluate the effects of flower abundance (log-scaled) on male and female components of reproductive success.

	Fitness component	Effect size	*F*-value	*p*-value
Male fitness	**Bee abundance (Log)**	**0.569**	**14.28**	**0.002**
	Pollen dispersal on bees (Log)	0.215	3.53	0.078
	Pollen bee disperser richness (bee richness)	**4.157**	**7.96**	**0.012**
Female fitness	Mean conspecific pollen deposition (Log)	0.137	0.87	0.365
	Pollen tube: Conspecific pollen ratio (Log)	−0.131	1.36	0.261

Note: Significant contrasts are shown in bold.

Muchhala, 2019). Preliminary evidence further suggests that the positive relationship between pollen protein content and mean pollen dispersal does not seem to be driven just by an increase in the size of bees visiting flowers with higher pollen protein levels. Our preliminary analyses showed no significant correlation between the average size of visiting bees and pollen protein content across plant species ($p > 0.05$; see Figure S3 for details). That is, plant species with higher pollen protein content were not visited by larger bees, suggesting pollen protein content increased the number of pollen grains dispersed irrespective of bee size. It is thus possible that resource investment in pollen nutritional quality (protein content) may primarily function to increase male reproductive success in plants; however, to our knowledge this possibility has remained unexplored. Animal-pollinated plants have been shown to have higher levels (and high phylogenetic signal) of pollen protein content compared to plants that do not depend on animal pollination (Ruedenauer et al., 2019). Higher pollen nutritional quality may be expected to attract not only pollinators but also other pollen consumers (Filipiak, 2016; Hargreaves et al., 2009). It is therefore important to note that trade-offs with non-pollinating pollen consumers (e.g. 'pollen thieves') could constrain the degree of investment in pollen protein content. Thus, optimal pollen protein content may be determined by a balance between increases in male reproductive success and pollen loss to non-pollinating insects. However, animal-pollinated plants could also use a combination of strategies (e.g. time of anthesis, poricidal anthers) and traits (e.g. floral chemistry, scent and other rewards) to minimize pollen loss and maximize reproductive gains via pollinivorous pollinators (e.g. Harder & Barclay, 1994; Hargreaves et al., 2009; Rivest & Forrest, 2020). Whether pollinators alone exert a sufficiently strong selective pressure on the nutritional profile of pollen, for instance compared to nectar (Parachnowitsch et al., 2019), to give rise to the large variation observed in this trait within plant communities remains to be determined. Thus, we emphasize the need for more studies on the evolutionary ecology of pollen nutritional quality (e.g. Filipiak et al., 2023; Parreño et al., 2022), particularly as it relates to plant fitness, in order to fully understand its role in mediating plan-pollinator interactions.

Interestingly, we did not observe a relationship between pollen protein content and any component of female reproductive success, suggesting the potential for sexual selection/conflicts driven by differential investment on this trait (i.e. differential allocation of resources to male and female functions; Chapman, 2006; Charnov, 1979; Parker, 1979; Willson, 1979). Conflict may arise if resource allocation to increase pollen protein content reduces the resources that can be allocated to ovule development (i.e. female fitness). Conflicts could also arise from sex-based fitness differences, if the amount of pollen protein required to attract pollinators versus the amount required for optimal pollen germination and pollen tube growth differ (Labarca & Loewus, 1973; Ruedenauer et al., 2020). In this sense, male and female functions may be subjected to different selection pressures with divergent evolutionary outcomes (Bergamo et al., 2024; Charnov, 1979; Ellis & Johnson, 2010; Moore & Pannell, 2011; Sutherland & Delph, 1984; Willson, 1979). For instance, pollen quantities and pollen/ovule ratios have been shown to be shaped by sexual selection, with high pollen production being advantageous for male fitness (Willson, 1979). Investment in male function has also been proposed as a factor influencing the evolution of different mating strategies, such as dioecy (separate male and female individuals) and dichogamy (temporal separation of male and female functions; Bawa, 1980; Charlesworth & Charlesworth, 1978; Thomson & Brunet, 1990; Willson, 1979). It is thus possible that investment in male function via increases in pollen nutritional quality may further contribute to the evolution of such mating strategies. Differential investment in male versus female functions can also depend on the probability of success of either function in a given environment (Bergamo et al., 2024; Charnov & Bull, 1977; Willson, 1979). The probability of pollen donation, pollen receipt and ovule maturation may vary across a range of biotic or abiotic conditions, or across plant species (Bergamo et al., 2024; Willson, 1979). This context-dependency may in turn contribute to the high within- (Buchmann, 1986; Cardoza et al., 2012; Yeamans et al., 2014) and among-species variation in pollen protein content observed (Vaudo et al., 2024), thus helping shape the diverse pollen nutritional landscape across local and global scales.

It is important to note that we did not observe a relationship between the number of bee morphospecies dispersing pollen of a given plant species and the pollen protein content of that same species. That is, pollen protein content did not affect floral visitor richness for a given plant species and this was only affected by flower abundance. Other studies have found similar results wherein pollen nutritional values were not related to overall estimates of plant–pollinator generalization (Vaudo et al., 2024), perhaps because not all pollinators depend on pollen as a food source (Ollerton, 2017). We specifically show that the richness of bee floral visitors, who strongly rely on pollen, is not affected by pollen nutritional quality (i.e. protein content). This could be due to varying bee preferences or needs for other types of macronutrients in pollen rewards. For instance, studies have highlighted the importance of lipids (Lau et al., 2022; Manning et al., 2007; Vanderplanck et al., 2011), vitamins (Mărgăoan & Cornea-Cipcigan, 2023) and nutrient ratios (Raubenheimer & Simpson, 1999; Vaudo et al., 2016) for pollinivorous insect pollinators. Bees, and other pollinating insects, can also show strong preferences for other types of floral rewards, especially nectar (Nicolson, 2022; Parachnowitsch et al., 2019; Venjakob et al., 2022). However, while most studies on pollen protein content levels have focused on its importance for bee fitness (Archer et al., 2021; Brunner et al., 2014; Greenberg, 1982; Kämper et al., 2016; Ren et al., 2023; Roger et al., 2017; Roulston & Cane, 2002; Schmidt et al., 1987), this study is one of the first to show that pollen protein content can have important ecological impacts on plants with unknown evolutionary consequences, particularly via its influence on male reproductive success.

Further, these results lend support to the prediction that survival versus reproduction trade-offs associated with plant life-history strategies contribute to variation in pollen protein levels in co-flowering communities. Specifically, we found higher pollen protein content in annual compared to perennial species. It is well-established that annual plants preferentially invest in reproductive (e.g. early flowering, large flower number and high-quality rewards) over survival traits (e.g. defence; Friedman, 2020; Vico et al., 2016). Our study suggests such trade-offs could also extend to affect the quality of rewards offered. Annual plants have also been shown to produce larger pollen volumes (Hicks et al., 2016), suggesting annual plants tend to maximize not only female but also male reproductive functions. This strategy may help reduce pollen limitation in annual compared to perennial species (Knight et al., 2005). Overall, our results show that differences in plant life-history strategies have the potential to contribute to the pollen nutritional landscape observed in diverse co-flowering communities (Vaudo et al., 2024), and that pollen nutritional quality may be an overlooked trait mediating levels of pollen limitation in nature.

Interestingly, we did not observe a phylogenetic signal on pollen protein content levels, suggesting pollen protein content in this serpentine seep community does not result from shared evolutionary history or shared metabolic needs (Labarca & Loewus, 1973; Ruedenauer et al., 2019). Congeneric species at our study sites, such as those in the *Clarkia* and *Allium* genera, did not have similar levels of pollen protein content (Figure 1). Meta-analytical studies, however, have found that plant species across larger phylogenetic trees can display a strong phylogenetic signal on pollen protein content (Ruedenauer et al., 2019). In contrast, at smaller spatial scales, pollen protein content has been shown to be plastic (Cardoza et al., 2012; Yeamans et al., 2014) and respond to variation in biotic and abiotic conditions (Russo et al., 2023; Ziska et al., 2016). Thus, high plasticity along with large effects on plant reproductive success and pollinator fitness may constrain phylogenetic effects on this trait at local scales; that is within single co-flowering communities. Overall, our study suggests the relative importance of different drivers of pollen protein content across species may vary across spatial and phylogenetic scales (Hanley et al., 2008; Roulston et al., 2000). Studies that further evaluate the ecological and evolutionary implications of variation in pollen quality in diverse co-flowering communities are needed to fully elucidate its importance for community assembly and the structure of plant–pollinator interactions.

AUTHOR CONTRIBUTIONS

Laura Russo, Liedson Tavares Carneiro, Gerardo Arceo-Gómez conceived and design the study, Jessica N. Williams, Daniel A. Barker, Anne Murray collected data in the field and in the laboratory. Laura Russo, Liedson Tavares Carneiro analysed the data and wrote the first draft. All other authors contributed significantly with revisions to the manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors have read and agreed to the submitted version of the manuscript and declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.2fqz61300 (Russo et al., 2024).

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REFERENCES

Alonso, C., Navarro-Fernández, C. M., Arceo-Gómez, G., Meindl, G. A., Parra-Tabla, V., & Ashman, T.-L. (2013). Among-species differences in pollen quality and quantity limitation: Implications for endemics in biodiverse hotspots. *Annals of Botany*, *112*(7), 1461–1469. https://doi.org/10.1093/aob/mct213

Arceo-Gómez, G., Abdala-Roberts, L., Jankowiak, A., Kohler, C., Meindl, G. A., Navarro-Fernández, C. M., Parra-Tabla, V., Ashman, T., & Alonso, C. (2016). Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization. *American Journal of Botany*, *103*(3), 396–407. https://doi.org/10.3732/ajb.1500155

Arceo-Gómez, G., & Ashman, T.-L. (2011). Heterospecific pollen deposition: Does diversity alter the consequences? *New Phytologist*, *192*(3), 738–746. https://doi.org/10.1111/j.1469-8137.2011.03831.x

Arceo-Gómez, G., & Ashman, T.-L. (2014a). Patterns of pollen quantity and quality limitation of pre-zygotic reproduction in *Mimulus guttatus* vary with co-flowering community context. *Oikos*, *123*(10), 1261–1269. https://doi.org/10.1111/oik.01309

Arceo-Gómez, G., & Ashman, T.-L. (2014b). Coflowering community context influences female fitness and alters the adaptive value of flower longevity in *Mimulus guttatus*. *The American Naturalist*, *183*(2), E50–E63. https://doi.org/10.1086/674358

Arceo-Gómez, G., Kaczorowski, R. L., & Ashman, T.-L. (2018). A network approach to understanding patterns of coflowering in diverse communities. *International Journal of Plant Sciences*, *179*(7), 569–582. https://doi.org/10.1086/698712

Archer, C. R., Fähnle, J., Pretzner, M., Üstüner, C., Weber, N., Sutter, A., Doublet, V., & Wilfert, L. (2021). Complex relationship between amino acids, fitness and food intake in *Bombus terrestris*. *Amino Acids*, *53*(10), 1545–1558. https://doi.org/10.1007/s00726-021-03075-8

Barrett, S. C. H., & Harder, L. D. (2017). The ecology of mating and its evolutionary consequences in seed plants. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 135–157. https://doi.org/10.1146/annurev-ecolsys-110316-023021

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bawa, K. S. (1980). Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics*, *11*(1), 15–39. https://doi.org/10.1146/annurev.es.11.110180.000311

Beattie, A. J. (1971). A technique for the study of insect-borne pollen. *The Pan-Pacific Entomologist*, *47*, 82.

Bergamo, P. J., Streher, N. S., Freitas, L., Wolowski, M., & Sazima, M. (2024). Male pollination success and female pollination success are mediated by opposing processes in a coflowering community. *International Journal of Plant Sciences*, *185*(2), 177–186. https://doi.org/10.1086/728235

Brunner, F. S., Schmid-Hempel, P., & Barribeau, S. M. (2014). Protein-poor diet reduces host-specific immune gene expression in *Bombus terrestris*. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20140128. https://doi.org/10.1098/rspb.2014.0128

Buchmann, S. L. (1986). Vibratile pollination in *Solanum* and *Lycopersicon*: A look at pollen chemistry. In *Solanaceae: Biology and systematics* (pp. 237–252). Columbia University Press.

Cardoza, Y. J., Harris, G. K., & Grozinger, C. M. (2012). Effects of soil quality enhancement on pollinator-plant interactions. *Psyche: A Journal of Entomology*, *2012*, 1–8. https://doi.org/10.1155/2012/581458

Carneiro, L. T., Williams, J. N., Barker, D. A., Anderson, J. W., Martel, C., & Arceo-Gomez, G. (2024). Patterns and drivers of pollen co-transport network structure vary across pollinator functional groups. *Journal of Ecology*, *112*, 2319–2332. https://doi.org/10.1111/1365-2745.14397

Chapman, T. (2006). Evolutionary conflicts of interest between males and females. *Current Biology*, *16*(17), R744–R754. https://doi.org/10.1016/j.cub.2006.08.020

Charlesworth, B., & Charlesworth, D. (1978). A model for the evolution of dioecy and gynodioecy. *The American Naturalist*, *112*(988), 975–997. https://doi.org/10.1086/283342

Charnov, E. L. (1979). The genetical evolution of patterns of sexuality: Darwinian fitness. *The American Naturalist*, *113*(4), 465–480. https://doi.org/10.1086/283407

Charnov, E. L., & Bull, J. (1977). When is sex environmentally determined? *Nature*, *266*(5605), 828–830. https://doi.org/10.1038/266828a0

Chau, K. D., & Rehan, S. M. (2024). Nutritional profiling of common eastern North American pollen species with implications for bee diet and pollinator health. *Apidologie*, *55*(1), 9. https://doi.org/10.1007/s13592-023-01054-4

Crone, M. K., & Grozinger, C. M. (2021). Pollen protein and lipid content influence resilience to insecticides in honey bees (*Apis mellifera*). *Journal of Experimental Biology*, *224*(9), jeb242040. https://doi.org/10.1242/jeb.242040

Dafni, A. (1992). *Pollination ecology: A practical approach*. IRL Press at Oxford University Press.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L., & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PLoS One*, *8*(8), e72016. https://doi.org/10.1371/journal.pone.0072016

Ellis, A. G., & Johnson, S. D. (2010). Gender differences in the effects of floral spur length manipulation on fitness in a hermaphrodite orchid. *International Journal of Plant Sciences*, *171*(9), 1010–1019. https://doi.org/10.1086/656351

Filipiak, M. (2016). Pollen stoichiometry may influence detrital terrestrial and aquatic food webs. *Frontiers in Ecology and Evolution*, *4*, 138. https://doi.org/10.3389/fevo.2016.00138

Filipiak, Z. M., Ollerton, J., & Filipiak, M. (2023). Uncovering the significance of the ratio of food K: Na in bee ecology and evolution. *Ecology*, *104*(8), e4110. https://doi.org/10.1002/ecy.4110

Freestone, A. L., & Inouye, B. D. (2006). Dispersal limitation and environmental heterogeinity shape scale-dependent diversity patterns in plant communities. *Ecology*, *87*(10), 2425–2432. https://doi.org/10.1890/0012-9658(2006)87[2425:DLAEHS]2.0.CO;2

Friedman, J. (2020). The evolution of annual and perennial plant life histories: Ecological correlates and genetic mechanisms. *Annual Review of Ecology, Evolution, and Systematics*, *51*(1), 461–481. https://doi.org/10.1146/annurev-ecolsys-110218-024638

Greenberg, L. (1982). Year-round culturing and productivity of a sweat bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society*, *55*(1), 13–22.

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*(4), 592–598. https://doi.org/10.1111/j.1365-2435.2008.01415.x

Harder, L. D., & Barclay, R. M. R. (1994). The functional significance of poricidal anthers and buzz pollination: Controlled pollen removal from *Dodecatheon*. *Functional Ecology*, *8*(4), 509. https://doi.org/10.2307/2390076

Hargreaves, A. L., Harder, L. D., & Johnson, S. D. (2009). Consumptive emasculation: The ecological and evolutionary consequences of pollen theft. *Biological Reviews*, *84*(2), 259–276. https://doi.org/10.1111/j.1469-185X.2008.00074.x

Hicks, D. M., Ouvrard, P., Baldock, K. C. R., Baude, M., Goddard, M. A., Kunin, W. E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Sinclair, F., Westbury, D. B., & Stone, G. N. (2016). Food for pollinators: Quantifying the nectar and pollen resources of urban flower meadows. *PLoS One*, *11*(6), e0158117. https://doi.org/10.1371/journal.pone.0158117

Jin, Y., & Qian, H. (2019). V. PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography*, *42*(8), 1353–1359.

Kämper, W., Werner, P. K., Hilpert, A., Westphal, C., Blüthgen, N., Eltz, T., & Leonhardt, S. D. (2016). How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. *Landscape Ecology*, *31*(10), 2245–2258. https://doi.org/10.1007/s10980-016-0395-5

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 467–497. https://doi.org/10.1146/annurev.ecolsys.36.102403.115320

Labarca, C., & Loewus, F. (1973). The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum*: II. Production and utilization of exudate from stigma and stylar canal. *Plant Physiology*, *52*(2), 87–92. https://doi.org/10.1104/pp.52.2.87

Lau, P., Lesne, P., Grebenok, R. J., Rangel, J., & Behmer, S. T. (2022). Assessing pollen nutrient content: A unifying approach for the study of bee nutritional ecology. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *377*(1853), 20210510. https://doi.org/10.1098/rstb.2021.0510

Leonhardt, S. D., & Blüthgen, N. (2012). The same, but different: Pollen foraging in honeybee and bumblebee colonies. *Apidologie*, *43*(4), 449–464. https://doi.org/10.1007/s13592-011-0112-y

Manning, R., Rutkay, A., Eaton, L., & Dell, B. (2007). Lipid-enhanced pollen and lipid-reduced flour diets and their effect on the longevity of honey bees (*Apis mellifera* L.). *Australian Journal of Entomology*, *46*(3), 251–257. https://doi.org/10.1111/j.1440-6055.2007.00598.x

Mărgăoan, R., & Cornea-Cipcigan, M. (2023). Carotenoids and vitamins of pollen. In N. Ecem Bayram, A. Ž. Kostic, & Y. Can Gercek (Eds.), *Pollen chemistry & biotechnology* (pp. 147–177). Springer International Publishing. https://doi.org/10.1007/978-3-031-47563-4_8

Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M., & Karron, J. D. (2009). New frontiers in competition for pollination. *Annals of Botany*, *103*(9), 1403–1413. https://doi.org/10.1093/aob/mcp062

Moore, J. C., & Pannell, J. R. (2011). Sexual selection in plants. *Current Biology*, *21*(5), R176–R182. https://doi.org/10.1016/j.cub.2010.12.035

Moreira-Hernández, J. I., & Muchhala, N. (2019). Importance of pollinator-mediated interspecific pollen transfer for angiosperm evolution. *Annual Review of Ecology, Evolution, and Systematics*, *50*(1), 191–217. https://doi.org/10.1146/annurev-ecolsys-110218-024804

Muchhala, N., Brown, Z., Armbruster, W. S., & Potts, M. D. (2010). Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, *176*(6), 732–743. https://doi.org/10.1086/657049

Muchhala, N., & Thomson, J. D. (2012). Interspecific competition in pollination systems: Costs to male fitness via pollen misplacement. *Functional Ecology*, *26*(2), 476–482. https://doi.org/10.1111/j.1365-2435.2011.01950.x

Nicolson, S. W. (2022). Sweet solutions: Nectar chemistry and quality. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *377*(1853), 20210163. https://doi.org/10.1098/rstb.2021.0163

Ollerton, J. (2017). Pollinator diversity: Distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 353–376. https://doi.org/10.1146/annurev-ecolsys-110316-022919

Parachnowitsch, A. L., Manson, J. S., & Sletvold, N. (2019). Evolutionary ecology of nectar. *Annals of Botany*, *123*(2), 247–261. 10.1093/aob/mcy132

Parker, G. A. (1979). Sexual selection and sexual conflict. In M. S. Blum & N. B. Blum (Eds.), *Sexual selection and reproductive competition in insects* (pp. 123–166). Academic Press.

Parreño, M. A., Alaux, C., Brunet, J.-L., Buydens, L., Filipiak, M., Henry, M., Keller, A., Klein, A.-M., Kuhlmann, M., Leroy, C., Meeus, I., Palmer-Young, E., Piot, N., Requier, F., Ruedenauer, F., Smagghe, G., Stevenson, P. C., & Leonhardt, S. D. (2022). Critical links between biodiversity and health in wild bee conservation. *Trends in Ecology & Evolution*, *37*(4), 309–321. https://doi.org/10.1016/j.tree.2021.11.013

Pirk, C. W. W., Boodhoo, C., Human, H., & Nicolson, S. W. (2010). The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). *Apidologie*, *41*(1), 62–72. https://doi.org/10.1051/apido/2009055

Radev, Z. (2019). The impact of protein content of pollen on its collecting by honey bees (*Apis mellifera* L.). *Modern Concepts & Developments in Agronomy*, *4*(2), MCDA.000583. https://doi.org/10.31031/MCDA.2019.04.000583

Raubenheimer, D., & Simpson, S. J. (1999). Integrating nutrition: A geometrical approach. *Entomologia Experimentalis et Applicata*, *91*(1), 67–82. https://doi.org/10.1046/j.1570-7458.1999.00467.x

Ren, C.-S., Chang, Z.-M., Han, L., Chen, X.-S., & Long, J.-K. (2023). Higher essential amino acid and crude protein contents in pollen accelerate the oviposition and colony foundation of *Bombus breviceps* (Hymenoptera: Apidae). *Insects*, *14*(2), 203. https://doi.org/10.3390/insects14020203

Rivest, S., & Forrest, J. R. K. (2020). Defence compounds in pollen: Why do they occur and how do they affect the ecology and evolution of bees? *New Phytologist*, *225*(3), 1053–1064. https://doi.org/10.1111/nph.16230

Roger, N., Michez, D., Wattiez, R., Sheridan, C., & Vanderplanck, M. (2017). Diet effects on bumblebee health. *Journal of Insect Physiology*, *96*, 128–133. https://doi.org/10.1016/j.jinsphys.2016.11.002

Roulston, T. H., & Cane, J. H. (2002). The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evolutionary Ecology*, *16*(1), 49–65. https://doi.org/10.1023/A:1016048526475

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*(4), 617–643. https://doi.org/10.1890/0012-9615(2000)070[0617:WGPCOP]2.0.CO;2

Ruedenauer, F. A., Raubenheimer, D., Kessner-Beierlein, D., Grund-Mueller, N., Noack, L., Spaethe, J., & Leonhardt, S. D. (2020). Best be(e) on low fat: Linking nutrient perception, regulation and fitness. *Ecology Letters*, *23*(3), 545–554. https://doi.org/10.1111/ele.13454

Ruedenauer, F. A., Spaethe, J., Van Der Kooi, C. J., & Leonhardt, S. D. (2019). Pollinator or pedigree: Which factors determine the evolution of pollen nutrients? *Oecologia*, *191*(2), 349–358. https://doi.org/10.1007/s00442-019-04494-x

Russo, L., Carneiro, L. T., Williams, J. N., Barker, D. A., & Arceo-Gomez, G. (2024). Data from: Differential effects of pollen nutritional quality on male and female reproductive success across plant species within a diverse co-flowering community [dataset]. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.2fqz61300

Russo, L., Ruedenauer, F., Gronert, A., Van De Vreken, I., Vanderplanck, M., Michez, D., Klein, A., Leonhardt, S., & Stout, J. C. (2023). Fertilizer and herbicide alter nectar and pollen quality with consequences for pollinator floral choices. *PeerJ*, *11*, e15452. https://doi.org/10.7717/peerj.15452

Russo, L., Vaudo, A. D., Fisher, C. J., Grozinger, C. M., & Shea, K. (2019). Bee community preference for an invasive thistle associated with higher pollen protein content. *Oecologia*, *190*(4), 901–912. https://doi.org/10.1007/s00442-019-04462-5

Schmidt, J. O., Thoenes, S. C., & Levin, M. D. (1987). Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. *Annals of the Entomological Society of America*, *80*(2), 176–183. https://doi.org/10.1093/aesa/80.2.176

Stanton, M., Young, H. J., Ellstrand, N. C., & Clegg, J. M. (1991). Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. *Evolution*, *45*(2), 268–280. https://doi.org/10.1111/j.1558-5646.1991.tb04402.x

Stebbins, G. L. (1950). *Variation and evolution in plants*. Columbia University Press. https://doi.org/10.7312/steb94536

Stephen, K. W., Chau, K. D., & Rehan, S. M. (2024). Dietary foundations for pollinators: Nutritional profiling of plants for bee health. *Frontiers in Sustainable Food Systems*, *8*, 1411410. https://doi.org/10.3389/fsufs.2024.1411410

Sutherland, S., & Delph, L. F. (1984). On the importance of male fitness in plants: Patterns of fruit-set. *Ecology*, *65*(4), 1093–1104. https://doi.org/10.2307/1938317

Thomson, J. D., & Brunet, J. (1990). Hypotheses for the evolution of dioecy in seed plants. *Trends in Ecology & Evolution*, *5*(1), 11–16. https://doi.org/10.1016/0169-5347(90)90006-Y

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*(10), 1807–1820. https://doi.org/10.1080/00032719.2010.526271

Vaudo, A. D., Dyer, L. A., & Leonard, A. S. (2024). Pollen nutrition structures bee and plant community interactions. *Proceedings of the National Academy of Sciences of the United States of America*, *121*(3), e2317228120. https://doi.org/10.1073/pnas.2317228120

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*(28), E4035–E4042. https://doi.org/10.1073/pnas.1606101113

Vaudo, A. D., Tooker, J. F., Patch, H. M., Biddinger, D. J., Coccia, M., Crone, M. K., Fiely, M., Francis, J. S., Hines, H. M., Hodges, M., Jackson, S. W., Michez, D., Mu, J., Russo, L., Safari, M., Treanore, E. D., Vanderplanck, M., Yip, E., Leonard, A. S., & Grozinger, C. M. (2020). Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects*, *11*(2), 132. https://doi.org/10.3390/insects11020132

Venjakob, C., Ruedenauer, F. A., Klein, A.-M., & Leonhardt, S. D. (2022). Variation in nectar quality across 34 grassland plant species. *Plant Biology*, *24*(1), 134–144. https://doi.org/10.1111/plb.13343

Vico, G., Manzoni, S., Nkurunziza, L., Murphy, K., & Weih, M. (2016). Trade-offs between seed output and life span—A quantitative comparison of traits between annual and perennial congeneric species. *New Phytologist*, *209*(1), 104–114. https://doi.org/10.1111/nph.13574

Wei, N., Kaczorowski, R. L., Arceo-Gómez, G., O'Neill, E. M., Hayes, R. A., & Ashman, T.-L. (2021). Pollinators contribute to the maintenance of flowering plant diversity. *Nature*, *597*(7878), 688–692. https://doi.org/10.1038/s41586-021-03890-9

Wei, N., Russell, A. L., Jarrett, A. R., & Ashman, T. (2021). Pollinators mediate floral microbial diversity and microbial network under agrochemical disturbance. *Molecular Ecology*, *30*(10), 2235–2247. https://doi.org/10.1111/mec.15890

Willson, M. F. (1979). Sexual selection in plants. *The American Naturalist*, *113*(6), 777–790. https://doi.org/10.1086/283437

Yeamans, R. L., Roulston, T. H., & Carr, D. E. (2014). Pollen quality for pollinators tracks pollen quality for plants in *Mimulus guttatus*. *Ecosphere*, *5*(7), 1–8. https://doi.org/10.1890/ES14-00099.1

Ziska, L. H., Pettis, J. S., Edwards, J., Hancock, J. E., Tomecek, M. B., Clark, A., Dukes, J. S., Loladze, I., & Polley, H. W. (2016). Rising atmospheric CO_2 is reducing the protein concentration of a floral pollen source essential for North American bees. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828), 20160414. https://doi.org/10.1098/rspb.2016.0414

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Taxonomic information and species codes for all 29 plant species studied.

Figure S1. Graphical representations of the non-significant relationships between pollen protein content and three measured components of plant reproductive success (see Table 1).

Figure S2. Relationship between plant species total flower abundance and (a) the number of flower-visiting bees (bee abundance) and (b) the number of bees carrying pollen from a given plant species (pollen disperser richness).

Figure S3. Association between pollen protein content and mean bee size, measured as the average of the intertegular distances across all flower visiting bees collected on each plant species.

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