

RESEARCH ARTICLE

Patterns and drivers of pollen co-transport network structure vary across pollinator functional groups

Liedson Tavares Carneiro¹  | Jessica Nicole Williams¹  | Daniel A. Barker¹  |
Joseph W. Anderson¹  | Carlos Martel²  | Gerardo Arceo-Gomez¹ 

¹Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee, USA

²Royal Botanic Gardens Kew, Richmond, UK

Correspondence

Liedson Tavares Carneiro
Email: liedson.tavares@gmail.com

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Abstract

1. The patterns and drivers of pollen transport on insect bodies can have important consequences for plant reproductive success and floral evolution; however, they remain little studied. Recently, pollinator bodies have been further described as pollen competitive arenas, where pollen grains can compete for space, with implications for the evolution of pollen dispersal strategies and plant community assembly. However, the identity, strength, and diversity of pollen competitive interactions and how they vary across pollinator functional groups is not known. Evaluating patterns and drivers of the pollen co-transport landscape and how these vary across different pollinator groups is central to further our understanding of floral evolution and co-flowering community assembly.
2. Here, we integrate information on the number and identity of pollen grains on individual insect pollen loads with network analyses to uncover novel pollen co-transport networks and how these vary across pollinator functional groups (bees and bee flies). We further evaluate differences in pollen load size, species composition, diversity and phylogenetic diversity among insect groups and how these relate to body size and gender.
3. Pollen co-transport networks were diverse and highly modular in bees, with groups of pollen species interacting more often with each other on insect bodies. However, the number, identity and frequency of competitors that pollen grains encounter on insect bodies vary between some pollinator functional groups. Other aspects of pollen loads such as their size, richness and phylogenetic diversity were shaped by bee size or gender, with females carrying larger but less phylogenetically diverse pollen loads than males.
4. *Synthesis.* Our results show that the number, identity and phylogenetic relatedness of pollen competitors changes as pollen grains travel on the body of different pollinators. As a result, pollinator groups impose vastly different interaction landscapes during pollen transport, with so far unknown consequences for plant reproductive success, floral evolution and community assembly.

KEYWORDS

functional traits, heterospecific pollen, phylogenetic diversity, plant–pollinator interactions, pollen competition, pollinator niche breadth, serpentine seeps

1 | INTRODUCTION

Pollination has been the subject of study for over 200 years (Darwin, 1877; Mayr, 1986; Vogel, 1996; Waser & Ollerton, 2006). As a result, much is known about the factors that influence pollen fate (Inouye et al., 1994), from pollen removal (e.g., pollen production and presentation) (Thomson & Thomson, 1992; Yeo, 1993), its deposition on stigmas (e.g., pollinator attraction and efficiency) (Armbruster et al., 2009; Harder & Routley, 2006; Inouye et al., 1994; Morales & Traveset, 2008; Moreira-Hernández & Muchhala, 2019), to pollen germination and ovule fertilization (e.g., stigma receptivity and pollen competition) (Arceo-Gómez & Ashman, 2011; Dafni & Maués, 1998; Lopes et al., 2022; Streher et al., 2020). Together, these represent the start and endpoints in the pollination process (Harder & Routley, 2006; Inouye et al., 1994), with implications for plant reproductive success (Gong & Huang, 2014; Johnson et al., 2005) and floral evolution (Barrett, 2003; Ellis & Johnson, 2010; Opedal et al., 2023). However, less is known about intermediate stages of pollen fate, such as the drivers, patterns, and consequences of pollen transport on insect bodies (but see Harder, 1990; Harder & Thomson, 1989; Minnaar et al., 2019 and references therein). Evaluating patterns of pollen transport on insect bodies can help uncover novel mechanisms affecting male and female success in plants (e.g., Cullen et al., 2021; Minnaar et al., 2019; Thomson & Thomson, 1989). It can also shed light into the evolution of pollen dispersal strategies (Anderson & Minnaar, 2020; Minnaar et al., 2019; Moir & Anderson, 2023) and inform on the potential for direct and indirect plant–plant interactions (e.g., competition) (Arceo-Gómez et al., 2016; Cullen et al., 2021; Lázaro et al., 2014). Recently, insect bodies have been described as ‘competitive arenas’, where co-transported pollen grains compete for space (Minnaar et al., 2019; Moir & Anderson, 2023), with potential evolutionary consequences. Nevertheless, the diversity, strength, and identity of pollen–pollen interactions, and how these vary across insects, is not well-known.

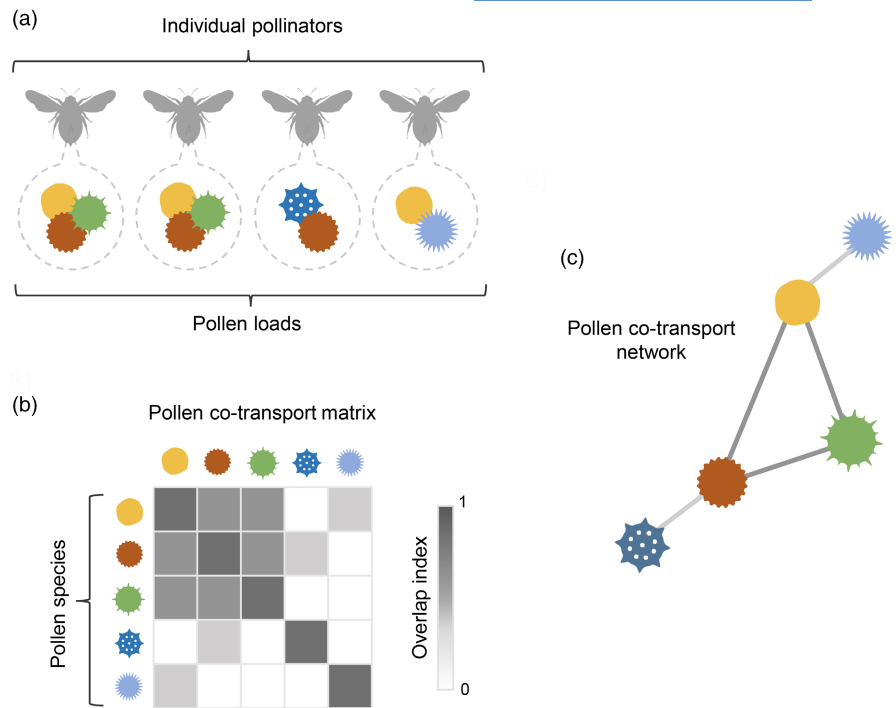
Knowledge of the size, diversity and identity of pollen loads carried by flower-visiting insects can be used to inform how pollinators differentially mediate ecological and evolutionary processes within communities. Patterns of pollen transport (load size and diversity) and co-transport (identity and frequency of co-transported pollen grains) may vary among insect species or taxonomic groups (Zhao et al., 2019) depending on their morphology, nutritional needs and foraging behaviour (Alarcón, 2010; Cullen et al., 2021; Vaudo et al., 2020). Bees, for example, are considered efficient at pollen transport due to their strong dependence on nectar and pollen (Ollerton, 2017; Zhao et al., 2019). Flies, on the other hand, are diverse in terms of body size, behaviour and pollen transport efficiency (Alarcón, 2010; Cullen et al., 2021; Zhao et al., 2019), although

their importance as pollinators has been well-documented, particularly hover flies (Syrphidae) and bee flies (Bombyliidae) (e.g., Cook et al., 2020; Larson et al., 2001; Orford et al., 2015). Even within bees, a wide range of morphologies and foraging behaviours likely lead to differences in patterns of pollen transport and co-transport. For instance, eusocial bees such as honeybees and bumblebees are known to have wide niche breadths (Johnson & Ashman, 2019; Kleinert & Giannini, 2012; Wood et al., 2021) resulting in large and diverse pollen loads. However, they may also represent harsher competitive arenas for pollen grains compared to solitary bees with narrower foraging niches (Grüter & Hayes, 2022).

Individual insect loads may be composed of one or very few pollen species even if pollinator species are considered generalists (Cane & Sipes, 2006; Leonhardt & Blüthgen, 2012; Smith et al., 2019; Tourbez et al., 2023). Foraging niche partitioning among individual insects within a species or taxonomic group may help generate differences in the identity and strength of interactions between pairs of pollen species; i.e., co-transport (Brosi, 2016; Smith et al., 2019). Evaluating patterns of pollen co-transport at the individual level is key not only for understanding differences in pollinator foraging niches and its implications for pollen transfer, but also provides insights on how pollinator species may generate different pollen–pollen interaction landscapes on their bodies (e.g., Minnaar et al., 2019). Here, we integrate information on the number and identity of pollen grains on individual insect bodies with network analytical tools to reveal detailed pollen co-transport networks (Figure 1) and evaluate how their structure varies across pollinator taxa. It is important to note that these networks provide evidence of realized patterns of pollen co-transport, that is pollen–pollen interactions that occur even when differences in habitat use and flowering time act to limit pollinator sharing and prevent pollen co-transport (Waser, 1978a, 1978b). This approach also helps identify groups of pollen species that travel and interact more often with each other (i.e., modularity; Olesen et al., 2007) and how these may change among different pollinators. Overall, this approach has the potential to further our understanding of how different pollinator groups can impose different ‘pollen competitive arenas’, influencing subsequent stages in the pollen pathway (Inouye et al., 1994; Minnaar et al., 2019).

Pollinator body size and gender can further help mediate differences in pollen transport among bees. Body size for instance can vary widely within and among bee species (Danforth, 2019; Michener, 2007), be directly related to foraging distance and pollen load size (Greenleaf et al., 2007; Wright et al., 2015), and influence the range of floral phenotypes that bees can access (Solís-Montero & Vallejo-Marín, 2017). Gender-based differences can also play an important role in shaping patterns of pollen transport (Cullen et al., 2021; Roswell et al., 2019). Unlike eusocial male bees, male solitary bees not only forage for nectar, but also use flowers for

FIGURE 1 Conceptualization and construction of pollen co-transport networks. (a) Pollen grains within pollen loads carried by individual insects within pollinator functional groups are identified and quantified. (b) A pollen co-transport matrix is created using Schoener's overlap index to quantify the magnitude of co-transport between pollen species. (c) A one-mode network is built with pollen species as nodes and overlap index values as weighted links.



patrolling and mating (Danforth, 2019; Pinheiro et al., 2017), potentially increasing the diversity of pollen loads. Females are also active pollen collectors and contact anthers more often compared to males (Danforth, 2019). In flies, particularly Bombyllidae species, differences in size between male and females (Knight, 1968) can influence their foraging patterns. Despite their influence on the size, diversity and composition of pollen loads, studies that evaluate how differences in body size and gender affect patterns of pollen transport and co-transport are scarce (see Panov, 2007).

Beyond their taxonomic identity and abundance, pollen grains can provide information on the evolutionary history of the plants transported by insects (Cullen et al., 2021; Wood et al., 2021). For instance, the phylogenetic diversity of pollen loads can provide information on key ecological and evolutionary process within co-flowering communities. Large phylogenetic diversity within pollen loads may suggest evolutionary convergence across plant species to attract similar pollinators (Wood et al., 2021), and/or niche differentiation among individual insects within a species or taxonomic group (e.g., generalist species; Smith et al., 2019). From an ecological perspective, pollen loads composed of closely related plant species (i.e., low phylogenetic diversity) may lead to stronger heterospecific pollen transfer effects (Arceo-Gómez et al., 2016; Arceo-Gómez & Ashman, 2011; Streher et al., 2020). High phylogenetic diversity in pollen loads may also indicate that insect bodies can act as competitive arenas for distantly related pollen grains, with unknown consequences for the evolution of pollen dispersal strategies.

In this study, we evaluate patterns of pollen transport (load size and diversity) and co-transport and how it varies among pollinator groups in a co-flowering community in Northern California. Particularly, we assessed how pollen load size, species composition,

richness, its phylogenetic assembly, as well as pollen co-transport network structure vary across bee and fly taxa. We further addressed whether differences in pollen load size, richness, and phylogenetic diversity are determined by body size and gender.

2 | MATERIALS AND METHODS

2.1 | Study site and system

The study was carried out at the McLaughlin Natural Reserve, Lower Lake, CA, USA (38°51'41.4" N, 122°23'54.8" W, alt. = ~650 m) during the peak flowering season of the serpentine seep metacommunity at two sites (BS and RHA; see Wei et al., 2021) in 2021 [Fieldwork permit was granted by the UC Natural Reserve System—Permit Application #40487]. Studied sites combined comprise ~50 herb and subshrub species growing within grasslands and shrublands (Koski et al., 2015), and ~200 flower-visiting insect species (Carneiro et al. unpublished data; also see Wei et al., 2021). The flowering season in the seeps is typically short, occurring between May and July (Alonso et al., 2017; Arceo-Gómez et al., 2018). Previous studies at these seeps have also shown the high importance of direct and indirect pollinator-mediated plant–plant interactions in mediating plant community assembly (e.g., Albor et al., 2020, 2022; Arceo-Gómez et al., 2016, 2018; Koski et al., 2015).

2.2 | Pollinator and pollen load sampling

Flower-visiting insects were collected foraging at the serpentine seeps between 09:00 and 15:00 h using entomological nets.

Collections took place each day by alternating sites over 13 days between May 9 and June 1, 2021. Insects were collected by 2–3 people simultaneously by walking at a steady pace while observing all plant species within each site and collecting every insect observed visiting a flower and contacting the reproductive structures or fully accessing floral tubes. After collection on flowers, specimens were immediately stored in tubes under freezing temperatures in the field to prevent insects from moving and minimize the loss of pollen. Pollen was collected from multiple insect body parts (head, proboscis, dorsal and ventral thorax, and fore- and mid-legs) using a single fuchsin jelly cube that was later mounted on a microscope slide (Barker & Arceo-Gomez, 2021; Beattie, 1971). The fuchsin jelly was made with 175 mL of distilled water, 150 mL of glycerol and 50 g of gelatin mixed with fuchsin crystals (Beattie, 1971). The jelly was cut into 3×3×1 mm cubes and then applied to all insect body parts. While pollen was obtained from all body parts, reliably assigning pollen to specific parts was not possible due to the small size of some insect groups (intertegular distance [ITD] range=0.69–4.81 mm). Insects were carefully handled with forceps to avoid pollen loss. Pollen carried on bee corbiculae or scopae was excluded because it generally represents a resource that is not directly available for pollination (Tong & Huang, 2018; Weinman et al., 2023), as the primary function of these structures is to collect pollen for larva provision (Danforth, 2019; Michener, 2007). This approach also helped standardize our sampling as pollen collecting structures are not present in flies and male bees. All pollen grains obtained from insects were counted under a microscope and identified based on a pollen library previously established from anthers collected for each plant species at the study sites. We obtained pollen load size, richness and composition for each individual insect.

Insects were separated into morphospecies and grouped into five major functional groups that represent their taxonomical identity as well as differences in their morphology and foraging behaviour, all of which can impact pollen transport (e.g., Cullen et al., 2021; Smith et al., 2019). The following pollinator groups were used: bumblebees, honey bees, megachilid bees, other bees (non-megachilid non-eusocial bees) and bee flies. Bumblebees and honey bees represent two different groups of eusocial bees, with well-recognized differences in size and foraging strategies and distances that can influence patterns of pollen transport and co-transport (Dornhaus & Chittka, 1999; Leonhardt & Blüthgen, 2012). Megachilid bees comprises a well-established taxonomic group of solitary bees whose representatives share a similar strategy of pollen collection and storage (Michener, 2007). The 'other bees' category is represented by multiple more distantly related species and is thus a more artificial group. However, these are included here as they still represent a frequent subset of flower visitors in the community (see Results) that are mainly non-eusocial and thus provide a valuable comparison with other more established pollinator groups in terms of their overall pollen load characteristics (size and diversity). Finally, bee flies comprise a specific and distinct

group of flower-visiting flies (Bombyliidae) that share similarities in morphology and foraging behaviour (Larson et al., 2001; Orford et al., 2015), and are also frequent flower visitors in the serpentine seep co-flowering communities (Cullen et al., 2021; Wei et al., 2021). These pollinator functional groups therefore include the most abundant pollinators in the seeps, accounting for 70% ($N=733$) of all insects collected at the studied sites. These groups have also been shown to be the most important pollen vectors in the serpentine seep metacommunities in previous studies (Albor et al., 2020, 2022; Cullen et al., 2021; Koski et al., 2015; Wei et al., 2021). Butterflies, for instance, represent less than 5% of all the floral visitors (also see Wei et al., 2021) and typically carry little to no pollen grains (12 pollen grains, on average), and thus were not considered in this study.

Bees and bee flies were separated into females and males, and the former were identified at the genus level using identification keys (Michener, 2007; Michener et al., 1994) prior to morphospecies assignment. We also measured bee ITD as a surrogate of body size (Cane, 1987). Insect specimens are preserved in the insect collection at the East Tennessee State University (ETSU).

2.3 | Pollen co-transport network

To evaluate differences in the structure of pollen co-transport networks across pollinator functional groups, we restricted our analysis to insects that were collected at the most well-sampled site. We used the number and identity of pollen grains on each individual insect to construct weighted pollen co-transport networks (Figure 1a–c) for each pollinator functional group, except 'other bees' as this group includes multiple unrelated species and thus an analysis at the individual level is less informative. To build pollen co-transport networks, we estimated the extent of 'insect body use' overlap between plant species (i.e., the magnitude of co-transport) (Figure 1b) whose pollen grains were observed on individual insects within each pollinator group (Figure 1c). This co-transport measure was calculated for each pollen species pair as the Schoener's niche overlap index (SI) (Linton et al., 1981; Schoener, 1970), which is widely used in ecological research (e.g., Albor et al., 2020, 2022; Arceo-Gómez et al., 2018; Forrest, 2015). Schoener's niche overlap index was estimated for each pollen species pair and within each pollinator functional group. A large value of pollen co-transport indicates a high magnitude of sharing of insect bodies between two pollen species within a pollinator group. We constructed one-mode networks using the *qgraph* R package (Epskamp et al., 2012) and the co-transport values (Schoener's niche overlap index) as weighted links between pollen species. For each pollen co-transport network, we estimated pollen co-transport degree (i.e., total number of co-transport partners) and strength (i.e., weighted degree representing the intensity of 'body use' overlap with other pollen species) for each plant species. We also estimated modularity (Q) to uncover groups of pollen species that travel more often with each other on insect bodies than with

other species within each pollen co-transport network using the optimal community structure algorithm through modularity maximization (Brandes et al., 2008).

2.4 | Phylogenetic diversity

We built a phylogenetic tree for each insect pollen load containing three or more plant species ($N=386$) using the function *phylo.maker* in the R package *V.PhyloMaker* (Jin & Qian, 2019), specifying the mega-tree of vascular plants 'GBOTB.extended' as the source tree (Smith & Brown, 2018). To estimate phylogenetic diversity of pollen loads transported by insects we obtained pairwise phylogenetic distances between plant species composing each pollen load using the function *cophenetic*. From each insect pollen load we computed the mean phylogenetic distance (MPD) adjusted by the proportional product of the abundances (i.e., number of pollen grains) of plant species presented in the matrix using the function *mpd* from the R package *picante* (Kembel et al., 2010; Webb et al., 2002).

2.5 | Data analyses

We modelled pollen load size, richness, and MPD as a function of pollinator category, gender and body size using generalized linear mixed models (GLMM). For pollen load size and richness, we fitted models for count data. Since models accounting for Poisson error-distribution were overdispersed, we fitted a negative binomial GLMM using *glmmTMB* (R package *glmmTMB*, Brooks et al., 2017), which improved all model parameters and fit. When modelling pollen load size and richness as a function of pollinator body size, we also considered its interaction with pollinator functional group to evaluate whether body size effects depend on pollinator group. For weighted MPD, we used Gaussian models using the *lmer* function (R package *lme4*, Bates et al., 2015). The variation clustered by insect samples belonging to the same morphospecies was considered by adding morphospecies identity as a random effect in all models. The proportion of variance attributed to the random variable morphospecies ranged from 11.9% to 78.5% (mean = 35.4%) in our models. To test for differences in the same response variables between female and male bees, we used a subset of our data containing only non-eusocial bees since all sampled bumblebees and honey bees were females. We conducted the same analyses considering female and male bee flies. We also tested whether pollen species co-transport degree and strength, extracted from pollen co-transport networks, varied among pollinator functional groups using GLMM with negative binomial and Gamma error-distributions, respectively. For both models, we included plant species identity as a random effect. Likelihood-ratio tests were used to evaluate the significance of the fixed effects when comparing the goodness of fit between the models and their respective null model. Post-hoc comparisons between pollinator groups were conducted with the

multcomp package (Hothorn et al., 2008). Finally, to compare species composition of pollen co-transport networks across pollinator functional groups, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using the *adonis2* function from the *vegan* package (Oksanen et al., 2013), followed by a Nonmetric Multidimensional Scaling (NMDS) with the *metaMDS* function to visualize (dis)similarities in pollen composition among groups. A Bray-Curtis distance similarity matrix was generated based on the composition of 274 pollen loads, which were used to generate NMDS coordinates. All data analyses were conducted in R v.4.2.3 (R Core Team, 2023).

3 | RESULTS

3.1 | Insect and pollen load diversity

In total, 733 flower-visiting bees ($N=710$) and bee flies ($N=23$) were collected at both studied sites (RHA=536, BS=197). Our samples represented 102 morphospecies, including 100 bee (29 genera) and two bee-fly morphospecies (Table S1). Bee samples comprised 51 bumblebees (five *Bombus* spp., mainly *Bombus vosnesenskii*), 71 honey bees, 219 megachilid bees (10 genera and 48 morphospecies), and 369 other non-eusocial bees (17 genera and 46 morphospecies) (Table S1). We collected 475 females and 113 males, disregarding eusocial bees, which are represented by female foragers only. Considering bee flies, 16 were females and seven were males. Overall, we counted 263,394 pollen grains from insect pollen loads representing 40 plant species (Table S2). Insects carried, on average, 359 pollen grains (median=86.5) and a mean richness equivalent to three plant species (mean=3.12, median=3). The most frequent plant species observed in pollen load samples were *Antirrhinum cornutum* (Plantaginaceae) (35.2%), *Streptanthus breweri* (Brassicaceae) (31.7%) and *Mimulus guttatus* (Phrymaceae) (26.1%). Pure pollen loads (i.e., only containing a single plant species) represented 17.6% of samples, mainly containing pollen from *Clarkia gracilis* (Onagraceae), *S. breweri*, *Eriophyllum lanatum* (Asteraceae) or *M. guttatus*, and were found in several morphospecies of bees (36). Only 6% of insect samples had no pollen grains. This percentage was also represented by different morphospecies of flower-visiting bees (25) and two bee-fly individuals.

3.2 | Variation in pollen transport components among pollinator groups

Pollinator functional groups differed in pollen load size ($\chi^2=10.93$, $df=4$, $p=0.027$; Figure 2a) and pollen load richness ($\chi^2=13.24$, $df=4$, $p=0.010$; Figure 2b), but not in MPD (phylogenetic diversity) ($\chi^2=5.27$, $df=4$, $p=0.260$). Specifically, bumblebees significantly transported larger pollen loads than megachilid bees. No other pollinator groups were significantly different from each other in terms of pollen load size (Figure 2a). Thus, bee flies exhibited similar pollen load size compared

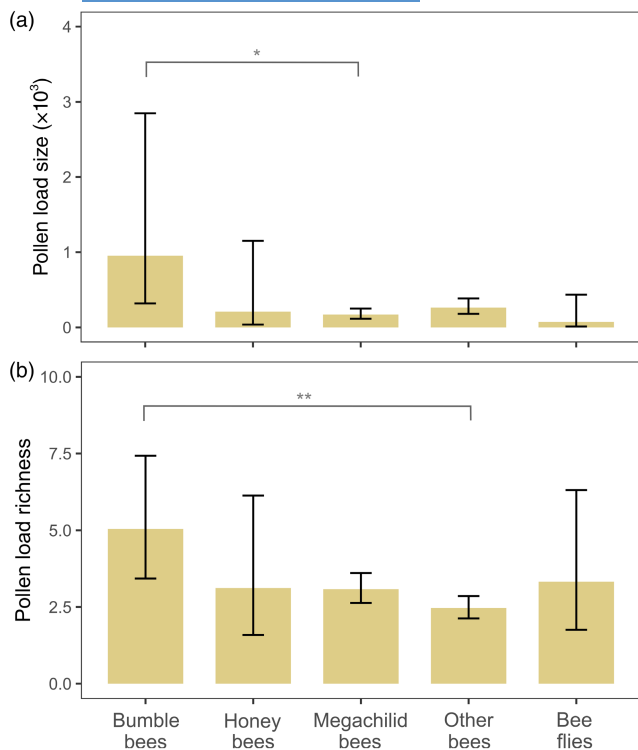


FIGURE 2 Least-squares means ($\pm 95\%$ CI) of pollen load size (a) and richness (b) obtained for each pollinator functional group in the co-flowering community at the serpentine seeps. Significance (p -value): $<0.010^{**}$, $<0.050^*$.

to all bee groups (Figure 2a). A larger pollen load richness was observed for bumblebees compared to other bees (Figure 2b). No differences in pollen load richness were found between honeybees, megachilid bees, other bees, and bee flies (Figure 2b).

3.3 | Pollinator body size and gender effects on pollen transport

Bee body size was positively associated with pollen load size ($\chi^2=11.57$, $df=4$, $p=0.021$; Figure 3a) and richness ($\chi^2=15.15$, $df=4$, $p=0.004$; Figure 3b), and these relationships did not vary with bee group (pollen load size: $\chi^2=1.00$, $df=3$, $p=0.802$; pollen load richness: $\chi^2=4.36$, $df=3$, $p=0.225$). In contrast, body size did not predict MPD ($\chi^2=1.25$, $df=1$, $p=0.263$).

Gender was also an important driver of pollen load size ($\chi^2=15.77$, $df=1$, $p<0.001$; Figure 4a), but not pollen load richness ($\chi^2=0.83$, $df=1$, $p=0.3$). Female pollen load size was more than two times larger compared to that of males (Figure 4a), even though pollen load richness was similar for both groups. However, males carried pollen loads with higher MPD than females ($\chi^2=4.17$, $df=1$, $p=0.041$; Figure 4b). No gender-based differences in studied pollen load components was found within bee flies (pollen load size: $\chi^2=1.48$, $df=1$, $p=0.224$; pollen load richness: $\chi^2=0.12$, $df=1$, $p=0.727$; MPD: $F=0.01$, $df=1$, $p=0.913$).

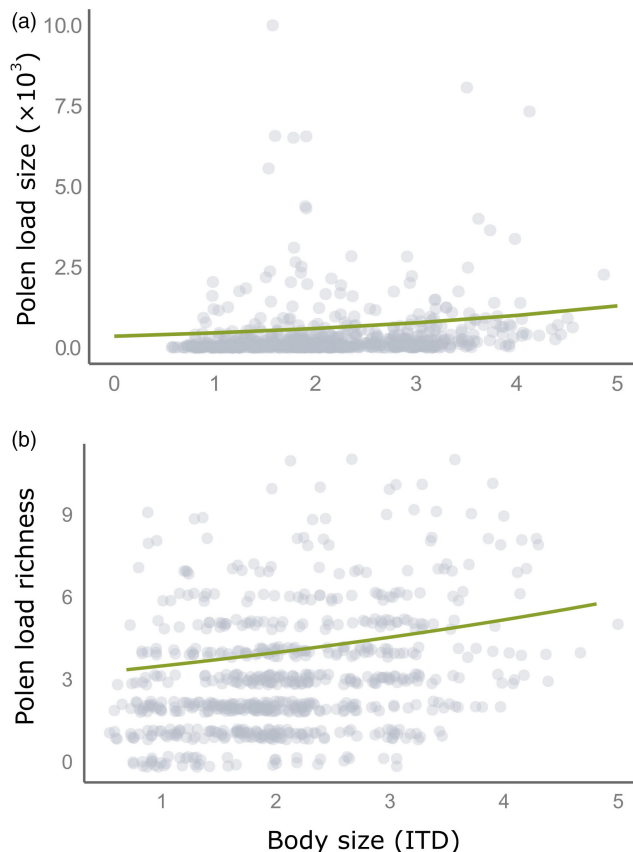


FIGURE 3 Effect of body size, measured as the intertegular distance of bees (ITD), on pollen load size ($p<0.001$) (a) and richness ($p=0.362$) (b) carried by bees at the serpentine seeps.

3.4 | Pollen co-transport networks of pollinator groups

We constructed pollen co-transport networks for 274 flower-visiting individual insects in four pollinator functional groups (excluding 'other bees') collected at the site with the highest sample size (RHA for bees and BS for bee flies) (Figure 5). We observed a total of 37 pollen species that were co-transported (i.e., shared an insect body). Co-transport networks contained between 18 and 32 pollen species across pollinator groups (bumblebees=29, honeybees=30, megachilid bees=32, and bee flies=18) (Figure 5). Pollen load composition differed among pollinator groups (PERMANOVA: $F=14.25$, $df=3$, $p=0.001$), with bee flies carrying different pollen species compared to all bee groups, which in turn transported similar species of pollen (Figure 6). The overall size of pollen co-transport networks (i.e., number of links) varied greatly among pollinator functional groups and ranged from 96 (bee flies) to 281 (megachilid bees). Network modularity also varied among pollinator functional groups ranging from 0.17 (bee flies) to 0.48 (bumblebees) indicating qualitative differences in the number of co-transport network modules formed across pollinators groups (bee flies=3, megachilid bees=4, honeybees=5 and bumblebees=6; Figure 5). Modules contained, on average, five to eight pollen species per pollinator group (Figure 5).

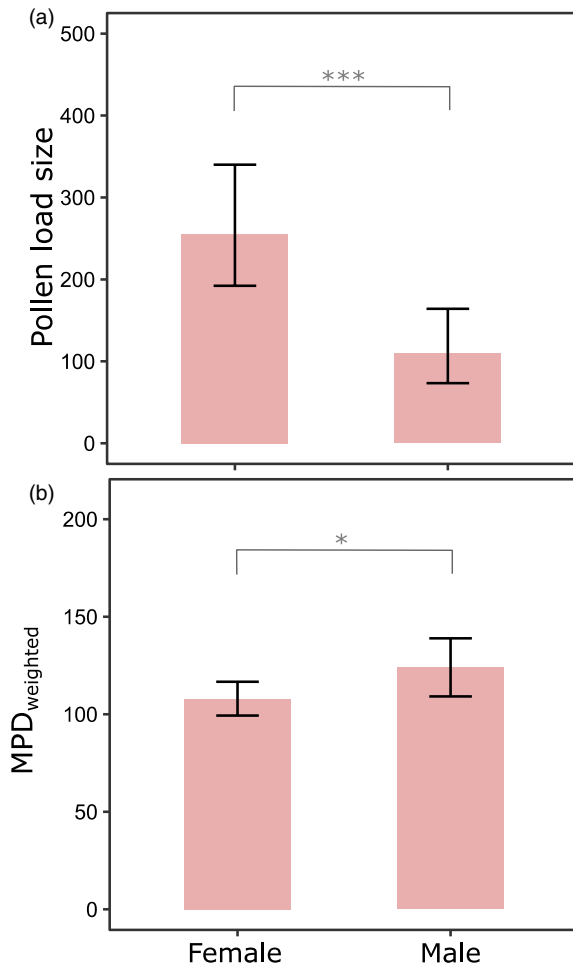


FIGURE 4 Least-squares means ($\pm 95\%$ CI) of pollen load size (a) and phylogenetic diversity (b), represented by the weighted mean phylogenetic distance (MPD_{weighted}) carried by female and male bees at the serpentine seeps. Significance (p -value): $<0.001^{***}$, $<0.050^*$.

Pollinator functional groups differed significantly in their pollen co-transport degree ($\chi^2 = 38.23$, $df = 3$, $p < 0.001$; Figure 7a) and co-transport strength (range = 0.04–3.58) ($\chi^2 = 9.43$, $df = 3$, $p = 0.025$; Figure 7b). Megachilid bees had a significantly higher pollen co-transport degree, indicating that pollen species carried by megachilid bees were co-transported with a larger number of other pollen species compared to those on bumblebees, honeybees, and bee flies (Figure 7a). Pollen species carried by bee flies were co-transported with a similar number of pollen species than those carried by bumblebees and honey bees (Figure 7a). However, pollen species travelling on honey bees showed lower co-transport degree than those observed on bumblebees (Figure 7a). Pollen co-transport strength was only different between honey bees and bee flies (Figure 7b).

4 | DISCUSSION

Individual insects carried on average between 7% and 12% (~3–5 species) of the total number of ‘pollen species’ (40 species) observed

in this study, suggesting a narrow but relatively generalized niche breadth at the individual level across pollinators (Smith et al., 2019; Tourbez et al., 2023). We also observed differences in patterns of pollen transport (load size, richness, species composition, MPD) and co-transport (co-transport network degree, strength, and modularity) among pollinator functional groups and between female and male bees, and hence in the potential for pollen–pollen interactions on insect bodies within (inter-individual) and between different pollinator groups. The ‘interaction landscape’ that pollen species face during transport can be further shaped by morphological, ecological and behavioural characteristics intrinsic to different pollinator groups (taxonomic identity, body size and gender). Our results provide evidence suggesting that different pollinator functional groups may represent different ‘pollen competitive arenas’ (sensu Minnaar et al., 2019), that may impose varying ecological and evolutionary pressures on pollen species as they travel on insect bodies.

At our study sites, bumblebees carried the largest and most diverse pollen loads compared to other pollinator groups. However, we also observed large within-group variation in pollen load size and richness in bumblebees. Large within-group variation may suggest differences in individual foraging that are not captured when evaluating group/species-level patterns, but that could play an important role in the structure of community-level plant–pollinator interactions (Brosi, 2016; Olesen et al., 2010; Tur et al., 2014). In fact, pollen co-transport networks revealed high modularity within bumblebees (six modules) and a large variation in the number of co-transported pollen species within each module (2–12 pollen species), suggesting a substructure of pollen–pollen interactions at the individual level within this pollinator group (Figure 5). This also suggest that while pollinator species can be generalist, individuals are more specialist (Tur et al., 2014) and may display higher levels of floral constancy (Grüter & Ratnieks, 2011; Waser, 1986). Nonetheless, bumblebees transported larger pollen loads compared to megachilid bees, and these were more species-rich compared to ‘other bees’ (non-megachilid non-eusocial bees), indicating a high relevance of bumblebees as pollinators (e.g., Goulson, 2006; Memmott et al., 2004). The high diversity of pollen loads carried by bumblebees, however, could also help strengthen plant–plant competitive interactions via heterospecific pollen transfer (Cullen et al., 2021; Morales & Traveset, 2008), with detrimental effects for plant fitness (Arce-Gómez, 2021; Morales & Traveset, 2008; Moreira-Hernández & Muchhala, 2019). Interestingly, the pollen load size and richness carried by honey bees was equivalent to that of any other pollinator group, revealing similar roles in pollen transport and reinforcing that honey bees may not be more important than other insect groups in their role as pollinators (e.g., Geslin et al., 2017; Magrath et al., 2017; Travis & Kohn, 2023). On the contrary, our results show that pollinator groups such as megachilid bees may play an underappreciated role as pollinators, as they carry comparable pollen load size to honey bees or even bumblebees in terms of pollen diversity and species composition. Bee flies also transport pollen loads similar in size and richness as some bee groups. Interestingly, bee flies transport pollen from a very distinct subset of plant species compared to bees,

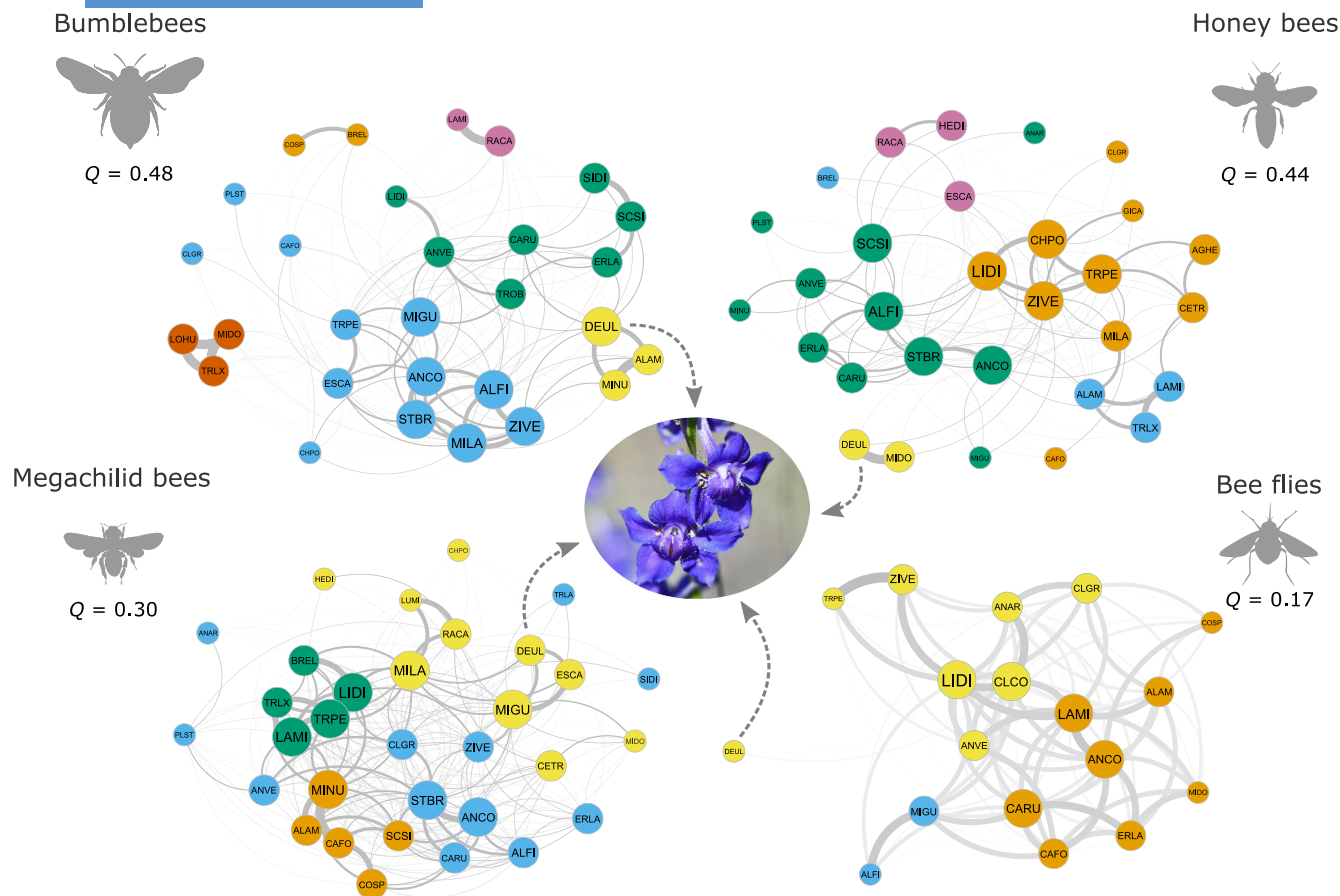


FIGURE 5 Pollen co-transport networks for four pollinator functional groups (bumblebees, honey bees, megachilid bees and bee flies). The modularity index (Q) for each network is shown. Each node represents a pollen species and its size represents the quantiles of pollen co-transport strength (weighted degree). Edge width represents the Schoener's insect body use overlap index (co-transport index) between pollen species-pairs. The different colours represent different pollen co-transport modules within each pollinator functional group. Note that *Delphinium uliginosum* (DEUL shown at the centre) travels with 1–9 other pollen species depending on the pollinator group. Species codes are noted inside each node and respective full species names are given in Table S2.

suggesting that these flies may be an important group of pollinators at our study sites, and perhaps in other highly diverse co-flowering communities (e.g., Kastinger & Weber, 2001).

Pollen co-transport networks showed that the number and identity of potential competitors that individual pollen species encounter on insect bodies (i.e., co-transport degree) varies across pollinator groups. Thus, pollinator functional groups likely represent different 'interaction landscapes' or 'competitive arenas' for individual pollen species. Differences in patterns of pollen co-transport occur even though these groups, particularly bees, share strong similarities in terms of the pollen load composition transported on their bodies. For instance, pollen species travelling on bodies of megachilid bees can encounter almost twice the number of pollen species (17 spp.), compared to those travelling on honey bees (8 spp.; Figure 7a), although these and other studied bee groups transport a similar subset of pollen species (Figure 6). Our co-transport network approach also showed that megachilid bees generate the highest diversity of pollen–pollen interactions, despite the fact that bumblebees transport the largest and most diverse pollen loads. This may result from differences in foraging niche partitioning among individuals within

each group, or it could also be due to the higher richness and abundance of megachilid bees sampled in the community compared to other pollinator functional groups. Nevertheless, it suggests that high pollen load richness at the level of pollinator species or functional group may not directly translate to a higher diversity of realized pollen–pollen interactions on insect bodies (e.g., Cullen et al., 2021), when patterns of pollen transport at the individual insect level are considered. Furthermore, our network approach revealed that some pollen species travel more often together, and thus may interact more frequently with each other on a pollinator's body (i.e., co-transport modules) (Figure 5). It further revealed that a single pollen species may face different 'interaction landscapes' (e.g., number and identity of pollen competitors) as it is transported by different pollinator groups. For example, the pollen of *Delphinium uliginosum* (Ranunculaceae) travels consistently with only one other pollen species when transported by honey bees (with *Minuartia douglasii*; Caryophyllaceae) or bee flies (with *Clarkia concinna*) (Figure 5). However, *D. uliginosum* travels with nine other pollen species on megachilid bee bodies, thus likely facing different competitive environments. This can also be evidenced in terms of mean frequency

FIGURE 6 Nonmetric multidimensional scaling (NMDS) plot showing (dis) similarities in pollen load composition transported by bumblebees, honey bees, megachilid bees and bee flies. A Bray-Curtis distance similarity matrix was calculated based on the composition of 274 pollen loads, which were used to generate NMDS coordinates. Ellipses indicate 95% confidence around the centroid of each pollinator functional group.

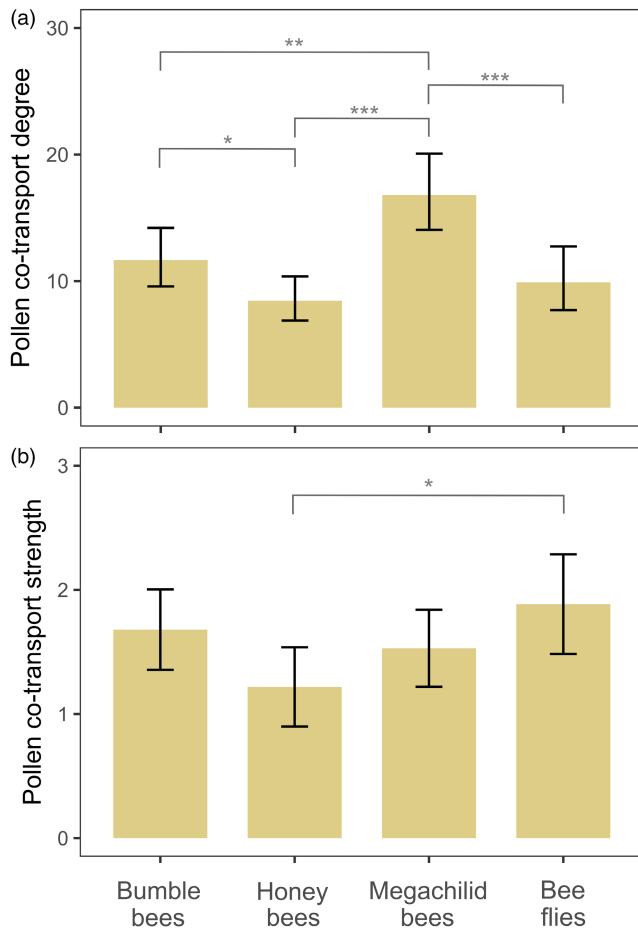
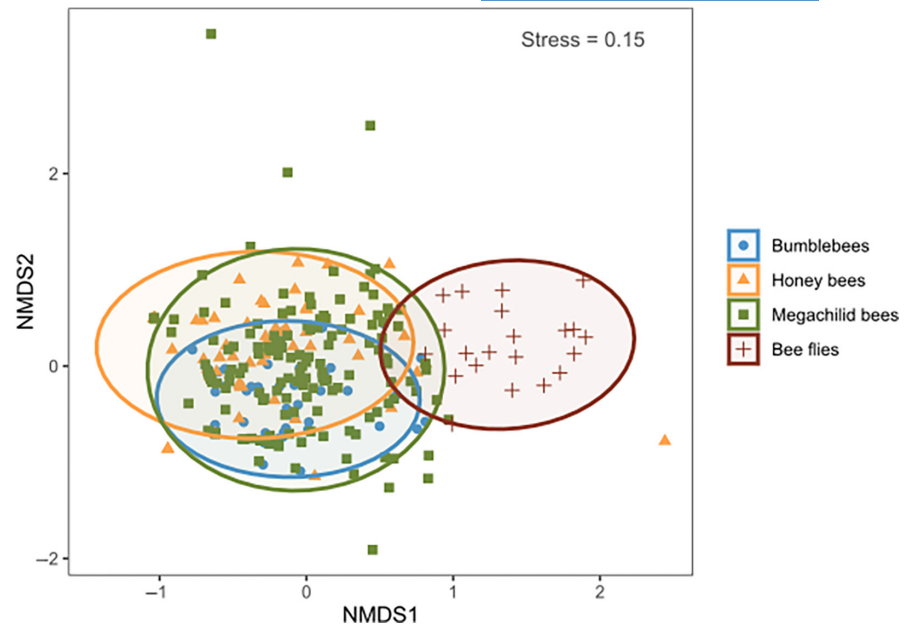


FIGURE 7 Least-squares means ($\pm 95\%$ CI) of pollen co-transport degree (a) that indicates the average number of co-transport partners of species per pollinator functional group in the co-flowering community, and pollen co-transport strength (weighted degree, b) which represents how frequent pollen species travel with others on the same individual pollinator. Significance (p -value): $<0.001^{***}$, $<0.010^{**}$, $<0.050^{*}$.

of pollen species encounters (i.e., pollen co-transport strength). On average, pollen species travel more often with each other on bee flies than on honey bees. Overall, our results suggest that differences in pollinator attributes and behaviours can lead to differences in the opportunity for plant species to interact and perhaps compete during pollen co-transport.

Differences in the pollen landscape on insect bodies (Minnaar et al., 2019) within and among different pollinator functional groups could thus have unforeseen ecological and evolutionary consequences. For instance, stronger competition for space on pollinator bodies could lead to the evolution of pollen weaponry (e.g., ornaments and chemicals) (Minnaar et al., 2019; Murphy, 2000), divergence on pollen placement (e.g., diffuse, stroke, stamp or layered) (Minnaar et al., 2019; Moir & Anderson, 2023; Morris et al., 1995) and/or dispersal strategies (e.g., sequential or vertical) (Harder & Wilson, 1998; Minnaar et al., 2019) that maximize siring success. If the consequences of these pollen interactions for male (e.g., Moreira-Hernández & Muchhala, 2019) and female fitness (e.g., Arceo-Gómez & Ashman, 2011) are strong, it may also lead to divergence or shifts in pollinator assemblages. It is important to note that the ultimate structure of pollen co-transport networks may be affected by other factors such as differences in flowering phenology that restrict certain interactions (e.g., forbidden links; Olesen et al., 2011), and differences in habitat use (environmental filters) and pollen placement on insect bodies (Huang et al., 2015; Tong & Huang, 2018). Although evaluating detailed patterns of pollen placement on insect bodies (Huang et al., 2015; Moir & Anderson, 2023) in diverse pollinator communities is challenging, such studies are a necessary step in order to fully understand the potential for pollen-pollen interactions as well as their ecological and evolutionary consequences. Evaluating the full complexity of pollen 'landscapes' on insect bodies within and among a diversity of pollinator functional groups is central for understanding their importance not only as pollinators, but also as mediators

of competitive plant–plant interactions via male (pollen competition on insect bodies) and female fitness (heterospecific pollen deposition).

Our results further revealed that pollen load characteristics can vary according to bee body size and gender. Pollinator body size has been widely recognized as a trait affecting foraging range (Greenleaf et al., 2007; Wright et al., 2015), floral fit (Agosta & Janzen, 2005; Solís-Montero & Vallejo-Marín, 2017) and pollen deposition on stigmas (Földesi et al., 2021). Here, we observed a positive relationship between body size and pollen load size and richness, also found in previous studies (Cullen et al., 2021; Smith et al., 2019). These results combined suggest that the importance of pollinators for plant reproductive success and as mediators of plant–plant interactions is dependent on body size. This is key as recent studies have found steep decreases in pollinator body size as a result of increasing temperatures (Herrera et al., 2023) and habitat disturbance (Fitzgerald et al., 2022; Grab et al., 2019; but see Warzecha et al., 2016). So far, the effects of warmer temperatures and habitat fragmentation on pollination have been mainly attributed to changes in phenology leading to plant–pollinator mismatches (Forrest, 2015) and pollinator declines (Klein et al., 2007; Potts et al., 2010). However, it is possible that warmer temperatures and habitat disturbances will also affect patterns of pollen transport and co-transport, pollinator efficiency and pollinator niche breadth via changes in insect body size, with unexplored consequences for the long-term persistence of plant communities.

Female bees carried twice the amount of pollen than males which may be explained by between-gender differences in foraging behaviour and ecological role (Ne'eman et al., 2006; Roswell et al., 2019). Whilst females have a more prominent and active role in pollen collection (Tang et al., 2019), males use flowers to feed on nectar, mate search and rest (Danforth, 2019; Eickwort & Ginsberg, 1980; Pinheiro et al., 2017). However, we found no differences in pollen load richness carried by male and females, in both bees and bee flies, suggesting a similar foraging niche breadth. Interestingly, in bees, pollen loads of males were more phylogenetically diverse than those of females despite similar pollen species richness, indicating differences in the evolutionary history and composition of the plants they visit (Roswell et al., 2019). Specifically, females visit a more closely set of related plants compared to male bees (also see Cullen et al., 2021), which can be, to some extent, a result of floral constancy or oligolectic levels that narrow foraging for pollen to certain related species in many solitary female bees (Cane & Sipes, 2006; Schindwein, 2004). In contrast, males carrying pollen of more distantly related species may be explained by the diversity of male activities on flowers other than foraging (Danforth, 2019), such as patrolling flowers in search of mates. This result, however, suggests that female-mediated heterospecific pollen transfer could have stronger detrimental consequences for plant fitness compared to male-mediated transfer. Although the evidence is still limited, some studies have suggested negative fitness effects of heterospecific pollen transfer can be stronger between closely related species (Arceo-Gómez &

Ashman, 2016; Streher et al., 2020). If this is the case, then male and female bees may impose differential heterospecific pollen transfer effects on the stigma driven by the different phylogenetic relatedness of pollen loads transported, a tantalizing possibility that remains unexplored.

Overall, our study highlights the importance of evaluating patterns of pollen transport beyond just pollen diversity and abundance, and that pollen co-transport networks can provide key insights that help inform on the variation in the identity and intensity of pollen–pollen interactions within and across pollinator groups. Our findings emphasize the importance of evaluating factors that affect intermediate stages in the pollination process, which are often overlooked and have the potential to inform on key ecological and evolutionary process within communities.

AUTHOR CONTRIBUTIONS

Liedson Tavares Carneiro and Gerardo Arceo-Gomez conceived the study and led the writing with substantial contributions by all authors. Jessica Nicole Williams, Daniel A. Barker and Gerardo Arceo-Gomez conducted fieldwork; Liedson Tavares Carneiro, Jessica Nicole Williams, Joseph W. Anderson and Carlos Martel conducted lab work; Liedson Tavares Carneiro led the analyses and result visualization. All authors gave final approval for publication. Our study brings together authors from a number of different countries, including scientists based in the country where the study was carried out.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare that are relevant to the content of this article.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14397>.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.hqbzkh1rp> (Carneiro et al., 2024).

ORCID

Liedson Tavares Carneiro  <https://orcid.org/0000-0002-4569-9500>

Jessica Nicole Williams  <https://orcid.org/0009-0007-9720-7903>

Daniel A. Barker  <https://orcid.org/0000-0002-6432-1810>

Joseph W. Anderson  <https://orcid.org/0009-0004-9026-3134>

Carlos Martel  <https://orcid.org/0000-0001-9892-1999>

Gerardo Arceo-Gomez  <https://orcid.org/0000-0003-3458-1600>

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SUPPORTING INFORMATION

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

Table S1. List of flower-visiting morphospecies of bees and flies and number of individuals per morphospecies sampled at the studied serpentine seep plant metacommunity in Northern California, USA, over 13 days between May 9 and June 1, during the peak flowering season in 2021.

Table S2. List of plant species found within pollen loads of the sampled flower-visiting insects at the studied serpentine seep plant metacommunity in Northern California, USA.

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