



The missing links: Bee and non-bee alpine visitor observation networks differ to pollen transport networks

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Abstract

1. A majority of the world's flowering plants benefit from insect pollination. Bees in particular are known to carry large amounts of pollen, and the pollen load transported is often highly conspecific. However, there is limited knowledge about the transfer of pollen by other non-bee flower-visiting insect taxa.
2. We observed and collected insects visiting flowers in an Australian alpine plant community. We identified insect body pollen loads to evaluate the relative differences among taxa using visitation and pollen transport networks. We sampled a diverse pollinator community from 39 insect families that visited 31 plant species ($n = 488$ individual insects).
3. Pollen abundance and richness on insect bodies varied significantly among Diptera, Hymenoptera and Lepidoptera both among individuals and across insect families.
4. Bees carried more pollen overall than the other three insect orders surveyed, yet dipterans were the most frequent flower visitors overall, with six dipteran families observed visiting flowers more frequently than the most frequent hymenopteran visitor (*Apis mellifera* L.). Apidae was also the only family in this study to carry consistently large quantities of pollen.
5. At the order level, Diptera carried the second highest quantity of pollen but greater diversity of pollen than other insect orders. Importantly, visitation networks revealed visits to plant taxa that were not identified in pollen transport networks and vice versa.
6. Given the missing links in both visitor observation and pollen transfer networks, we advocate combining both types of networks to provide a more accurate estimate of the full range of plant-pollinator interactions occurring within and across taxa at the community level. Understanding the variation in plant-pollinator interactions as a result of differences among taxa and between networks of flower visitors, pollen transfer is important to evaluate the level of generalisation/specialisation among plants and their pollinator partners.

KEYWORDS

alpine ecology, Diptera, heterospecific pollen, pollen transfer, pollination network, response diversity

Francisco Encinas-Viso and Emma Goodwin contributed equally to this study.

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INTRODUCTION

Many flowering plants depend on animal pollination, which involves pollen from the same species of plant (i.e., conspecific pollen) being transferred from male to female flower parts in order to produce viable seed (Faegri & Van Der Pijl, 1979; MacPhail et al., 2018). Insect pollinators are by far the most common animal pollen transport vector, yet the quality and quantity of conspecific pollen transferred vary among insect taxa (Bischoff et al., 2013; Fang & Huang, 2013). The constancy exhibited by bees is thought to result in the transport of large amounts of conspecific pollen (Alarcón, 2010; Barrios et al., 2016; Lefebvre et al., 2014; Willmer et al., 2017). Yet, in taxa other than bees, little experimental evidence exists that relates the relative amounts of conspecific and heterospecific pollen carried (Alarcón, 2010).

Quantifying the amount and type of heterospecific (or foreign) pollen carried on insects is important as it is known to play an important role in determining plant fitness and community assembly as more foreign pollen generally reduces seed production (Arceo-Gomez et al., 2018; Fang & Huang, 2016; Flanagan et al., 2009; Mitchell et al., 2009; Morales & Traveset, 2008). Few studies have assessed the differences among flower-visitor taxa in their contribution to heterospecific pollen transfer (Alarcón, 2010) and differences among individuals of the same pollinator taxon (Tur et al., 2014). Some moths are known to exhibit floral fidelity (Devoto et al., 2011), whereas other taxa such as flies are thought to be more variable (Inouye & Pyke, 1988; Lefebvre et al., 2018). Because of the complexities relating to pollen transfer by different taxa, we still lack a general understanding of the broader issue of generalisation and specialisation in plant–pollinator interactions, and studies of this kind are often subject to observational biases based on assumed knowledge (Armbruster, 2017; Lefebvre et al., 2018; Willmer, 2011).

Here, we observed and then captured flower-visiting insects across a range of diverse plant communities in the Australian alpine zone, where non-bees are important visitors (Goodwin et al., 2021; Inouye & Pyke, 1988). We observed bee and non-bee flower visitors to produce flower visitation networks and then identified the captured insects' pollen loads to directly compare floral visitation networks to pollen transfer networks (King et al., 2013; Tur et al., 2014).

Specifically, we asked the following questions:

- i. How do bee and non-bee taxa differ with respect to body pollen load composition (i.e., amount of conspecific and heterospecific pollen)?
- ii. Are particular plant and pollinator taxa overlooked (i.e., identified as 'missing links') when either floral visitation or pollen transport networks are utilised in isolation? and
- iii. How does the variation among individuals and families influence node strength (NS) in pollen transfer networks?

METHODS

Study sites and design

Fieldwork was undertaken with permission from National Parks and Wildlife Service under Scientific Licence no. SL101958. Sampling took

place at 12 plots at Mt Stilwell, Charlotte Pass, Kosciuszko National Park, New South Wales (−36.4326° N, 148.3278° W) in the austral summer January 2018 during peak flowering for the area. The flowering season for the region is November to March, with January being the peak of flowering with maximum floral diversity recorded in previous studies (Costin et al., 2000; Inouye & Pyke, 1988). The lowest temperature recorded in January 2018 at the site was −0.2°C, and the highest temperature was 27.6°C (Bureau of Meteorology, 2018). Minimum temperature during sampling sessions was 6.5°C, and the maximum was 26.2°C. Mountains and ridges are often subject to high winds with an average of 6.4 m/s during January. Average annual precipitation for the area is 1430 mm (Bureau of Meteorology, 2018).

We used stratified sampling within different habitat types to include three replicates of four different vegetation communities (12 sites total): (1) subalpine woodland/heath (mid-slope), (2) alpine herbfield/grassland (mid-slope), (3) alpine heath/bogs and fen (upper slope), and (4) alpine herbfield/fjaldmark (ridge). These communities were based on 'Keith' vegetation classifications (Costin et al., 2000).

Visitor observations

To investigate the frequency and identity of visiting insect taxa, we carried out floral-visitor observations. We observed flowering plant species within each plot for 30-min periods. Each plot was sampled six times. This equated to 3 h of sampling per plot between 10 AM and 4 PM and 36 h of observations in total. The order of plots for sampling was randomised to avoid one site being sampled at the same time every day.

At the beginning of each observation, environmental data were collected from 1 m above the ground (temperature, humidity and average wind speed) using a Kestrel anemometer (Kestrel 2000 Model). During each sampling session, the same observer recorded and collected flower visitors using a sweep net. The observer walked transects through the quadrat, stopping for approximately 5-min intervals at flowering plants and capturing and recording any insect visitors. Only the insects observed to touch the reproductive parts of the flower were collected as these were considered potential pollinators. For insects that were common and identification was known, the number of individuals was recorded, and the insects were not collected to minimise destructive sampling (e.g., *Apis mellifera* L., *Chauliognathus lugubris* F.).

Insect collection for pollen transfer network

To understand how the pollen load and richness of pollen (i.e., number of morphospecies) varied across taxa, we sampled three insect orders (Diptera, Lepidoptera and Hymenoptera) known to be common pollinating insect taxa in other alpine environments (Brown & McNeil, 2009; Johanson et al., 2018; Lefebvre et al., 2014). Coleoptera were excluded as the majority of visitors observed were large mating aggregations of the plague soldier beetle (Cantharidae: *C. lugubris*), and most individuals were not observed moving between plants. Ants were also excluded from analysis as preliminary

observations demonstrated little movement between plants and they are not considered pollinators in some systems (Beattie, 2006; Beattie et al., 1984, but see Natsume et al., 2022, Gras et al., 2016 and Das & Das, 2023). We hereafter refer to Hymenoptera as bees and wasps.

Insects collected from the visitor observations were kept frozen until pollen was removed from insect bodies using a basic fuchsin jelly (Kearns & Inouye, 1993). Corbiculae (pollen baskets) from *A. mellifera* (which is the only bee with corbiculae in our study) were avoided as pollen from corbiculae is unlikely to be available for pollination (Kearns & Inouye, 1993; Stavert et al., 2016). The fuchsin gel was melted onto microscope slides, staining the pollen. Forceps were rinsed with hot water in between each insect to minimise pollen transfer/contamination between insects and dried with clean paper towel.

A total of 122 slides stratified across insect order were selected randomly to count and identify pollen grains using a microscope (Leica ICC50W) at $\times 10$ to $\times 100$ zoom. This subsample consisted of 40 slides each for Hymenoptera and Lepidoptera and 42 slides for Diptera. For each of the subsampled slides, a 20×20 mm adhesive slide grid was placed on the underside of the slide to minimise miscounts. Pollen grains were identified to family level with use of a reference slide collection made from plant material collected at the study site and compared with images available online from the Australasian Pollen and Spore Atlas (APSA Members, 2007) and the Global Pollen Project databases (Martin & Harvey, 2017). Because of our inability to conclusively distinguish among plant species with light microscopy and available databases, we identified pollen to the family level. In the pollen transfer networks, we thus refer to 'con-familial pollen' if its body pollen was from the same plant family that the insect was captured upon and 'hetero-familial pollen' if the pollen carried was from one or more different plant families.

Collected insects were mounted and identified to family or morphospecies by invertebrate specialists at the Australian National Insect Collection (CSIRO, Black Mountain, Australian Capital Territory) (see Table S4). All analyses were run on family-level identification. Samples are deposited in the Australian National Insect Collection (CSIRO, Black Mountain, Australian Capital Territory).

Data analysis

To investigate the variation in pollen load composition and quantity carried by different insect taxa, we plotted the number of observations of each insect family to illustrate which taxa were the dominant visitors among the sample orders. This was visualised using a bar plot created by 'geom_barplot' function in the R package 'ggplot2' (Wickham, 2009). We examined the variability among pollen load and richness using boxplots created with the 'geom_boxplot' function in 'ggplot2' showing the median and interquartile ranges of the data (Wickham, 2009). The two Kruskal–Wallis analyses of significant difference between number and richness of pollen grains across orders were undertaken using the 'kruskal.test' function and adjusted *p*-values using Bonferroni method for multiple test comparisons in R 4.2.1 (R Core Team, 2018).

To determine differences in the quantity and proportions of pollen carried by the three insect orders, we ran generalised linear mixed

models (GLMMs) with total pollen, total pollen richness, amount of con-familial pollen and amount of hetero-familial pollen as the response variables and order as the predictor, with site as a random effect. We used the 'glmer' function in package 'lme4' (Bates et al., 2015) in R 4.2.1 (R Core Team, 2018). Response variables were over-dispersed count data, and we used a negative binomial error structure for all models. We validated this was an appropriate fit by comparing AIC values with the Poisson model for each one. To increase the robustness of our results, we only used insect families that had three or more specimens per morphospecies for all statistical analyses.

To identify plant and pollinator taxa that may be overlooked by using different methods (visitor observations and palynological studies), we constructed bipartite networks connecting taxonomic families of flowering plants with the taxonomic orders of insect pollinators observed visiting them. Data from both networks (visitation and pollen data) were pooled to construct a visitation–pollen network with missing links. This network also included observations of flower visitors (pollinators) that were not carrying pollen. Missing links were calculated as links or plant–pollinator interactions not recorded on the visitation network, but present on the pollen-based network. Finally, NS (Dormann, 2011) was calculated for family-level and individual-level pollen transfer networks to compare the relative importance of each taxon within the network. We calculated NS as an indicator of importance of each individual in the network as it identifies key individuals in the network that plant species may depend on. High NS in a network weighted by pollen abundance reflects those insects carrying the highest number of pollen grains. To calculate NS, we used the function 'strength' from the 'bipartite' (Dormann et al., 2008) package in R 4.2.1 (R Core Team, 2018).

All network analyses and visualisations were conducted in packages 'colorBlindness', 'igraph' (Csardi & Nepusz, 2006) and 'bipartite' (Dormann et al., 2008) in R software version 4.2.1 (R Core Team, 2018). The silhouette images of insects in figures were acquired from PhyloPic [phylopic.org; Creative Commons Credits: Fly, Beetle (Gareth Monger); Bee (Melissa Broussard); Butterfly (public domain)].

RESULTS

Visitor observations

In total, we observed 488 insects across three orders (Diptera, Hymenoptera and Lepidoptera) and 39 insect families visiting the flowers. From the insects collected, 28 morphospecies across the three orders had only one representative specimen (Table S1). From these orders, Diptera (flies) were the most frequent flower visitors overall and had the highest richness of visiting families (74.2%, $N = 362$, 18 families). Lepidoptera (butterflies/moths) (14.3%, $N = 70$, 10 families) was the next most frequent order. Hymenoptera (bees/wasps) (11.5%, $N = 56$, 11 families) was the least frequent order (Figure S1). The dominating insect families were Calliphoridae and Tachinidae from the Diptera order (Table S1). The insect community was recorded

visiting 27 different plant species from 9 plant families. The visitation observations showed that Asteraceae (14 species) was the most frequently visited plant family (51.0% of all recorded visits), followed by Myrtaceae (15.4%) and Ericaceae (11.4%).

Pollen grains carried by different taxa

A total of 24,406 pollen grains were counted on the subsampled 122 insects. Hymenoptera carried the greatest quantity of pollen grains out of the three orders ($N = 13,588$, 55.7% of the total grains counted). Diptera carried the next highest quantity of grains overall ($N = 7358$, 30.1%), and Lepidoptera carried the least ($N = 3460$, 14.2%). Hymenoptera had an average of 340 (± 50.9) pollen grains per insect followed by Diptera (175 ± 34.6 grains per slide) and Lepidoptera (84 ± 18.5 grains per slide) (Figure S2). There were significant differences of amount pollen carried among insect orders ($\chi^2 = 8.65$, $df = 2$, $p < 0.01$). Hymenoptera carried significantly more pollen grains than Lepidoptera ($Est = -2.94$, $p < 0.001$). However, Diptera did not carry significantly more pollen grains than Hymenoptera ($Est = -1.56$, $p > 0.05$) nor Lepidoptera ($Est = 1.39$, $p > 0.05$).

All insect orders showed relatively high variability in pollen load. Although Diptera on average had a lower pollen load than Hymenoptera, some dipterans carried up to the maximum pollen load counted in this study (700 grains) (Figure S2). Similarly, all orders had individuals that carried very few pollen grains (under 20) or did not carry any pollen grains.

There were no statistically significant differences in pollen richness carried among insect orders ($\chi^2 = 5.75$, $df = 2$, $p > 0.05$). However, Diptera and Hymenoptera tended to carry pollen from two to five plant families, and GLMM analysis shows that Lepidoptera tended to carry significantly less types of pollen ($Est = -0.30855$, $p < 0.05$),

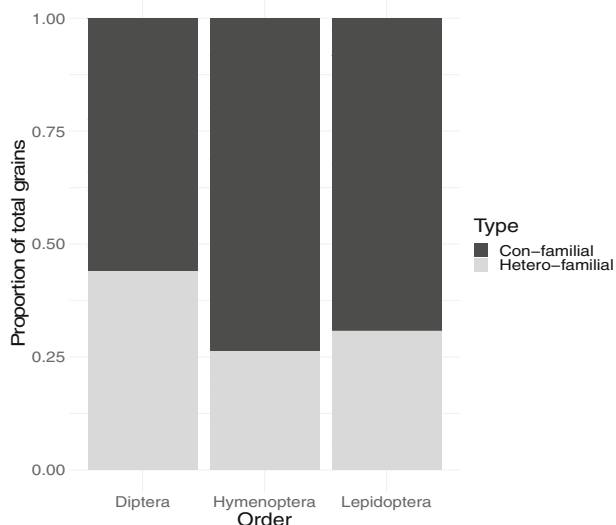


FIGURE 1 Overall proportion of con-familial and hetero-familial pollen grains for each order. Black colour shows con-familial and grey hetero-familial proportion of pollen grains.

with most insects carrying pollen from one to three plant families (Figure S3). There were no significant differences of con-familial ($\chi^2 = 4.1231$, $df = 2$, $p > 0.1$) nor hetero-familial ($\chi^2 = 4.1231$, $df = 2$, $p > 0.1$) pollen grains carried among insect orders.

Although Lepidoptera carried the least pollen, they had the highest overall proportion of con-familial pollen from the three orders (Figure 1 and Figure S4). Lepidoptera carried a slightly higher average proportion of con-familial pollen than Hymenoptera with 85.0% of pollen carried by Lepidoptera being con-familial (Figure 1). Hymenoptera carried an average of 84.4% of pollen matching the visited plant family. Diptera carried the least con-familial pollen of the three orders, but this was nonetheless quite high with an average of 73.9% con-familial pollen. Diptera tended to carry more hetero-familial pollen (26.1%) than the other orders, and Lepidoptera carried the least (15.0%) (Figure 1).

Combining visitor observations and pollen transport networks

The visitors in the pollen transport network visited Asteraceae most frequently, followed by Orobanchaceae (*Euphrasia collina* R.Br.), Ericaceae and Myrtaceae. Of all insects sampled, we found that the highest abundance of pollen grains was also from Asteraceae (76.0% of all plant families recorded). Asteraceae and Myrtaceae pollen occurred more frequently in the pollen transport network than in the visitor observations. However, insects observed on other frequently visited plant families (such as Orobanchaceae, Lamiaceae and Ericaceae) shown in the visitation network (Figure S4a) carried limited pollen as shown in the pollen transfer network (Figure S4b). Orobanchaceae pollen was not frequently observed in the pollen transport network, and only Hymenoptera and Diptera were found to carry this pollen. However, insects from all three orders were captured on plants from this family (Figure S5).

Apidae (all *A. mellifera* L.) was the only family to consistently carry large quantities of pollen and generally of a single type; 12 out of 14 Apidae individuals sampled carried large amounts of Asteraceae pollen (Figure 2). At an individual level, more links were observed in the pollen transfer network than captured collectively in the visitor observations (Figure 2 and Figure S6). However, the missing link analyses revealed few taxa recorded in the visitor observation network that were not identified in the pollen transfer network, but many taxa recorded in the pollen transfer network that were not observed in the visitation network. For example, Braconidae (Hymenoptera) was only observed visiting Asteraceae but carried pollen from Ericaceae and Myrtaceae (Figure 3a). More specifically, we found a total of 100 missing links, from which 31 were for Hymenoptera, 37 from Diptera and 32 from Lepidoptera. The plant families who had the largest number of missing links were Lamiaceae (25) and Myrtaceae (15) (Figure 3). In addition, all pollinator taxa had missing links, that is, visitation data did not provide the same information as pollen data for any pollinator taxa.

At the resolution of family, Apidae (for this study, all *A. mellifera*) consistently carried large amounts of pollen, usually from Asteraceae

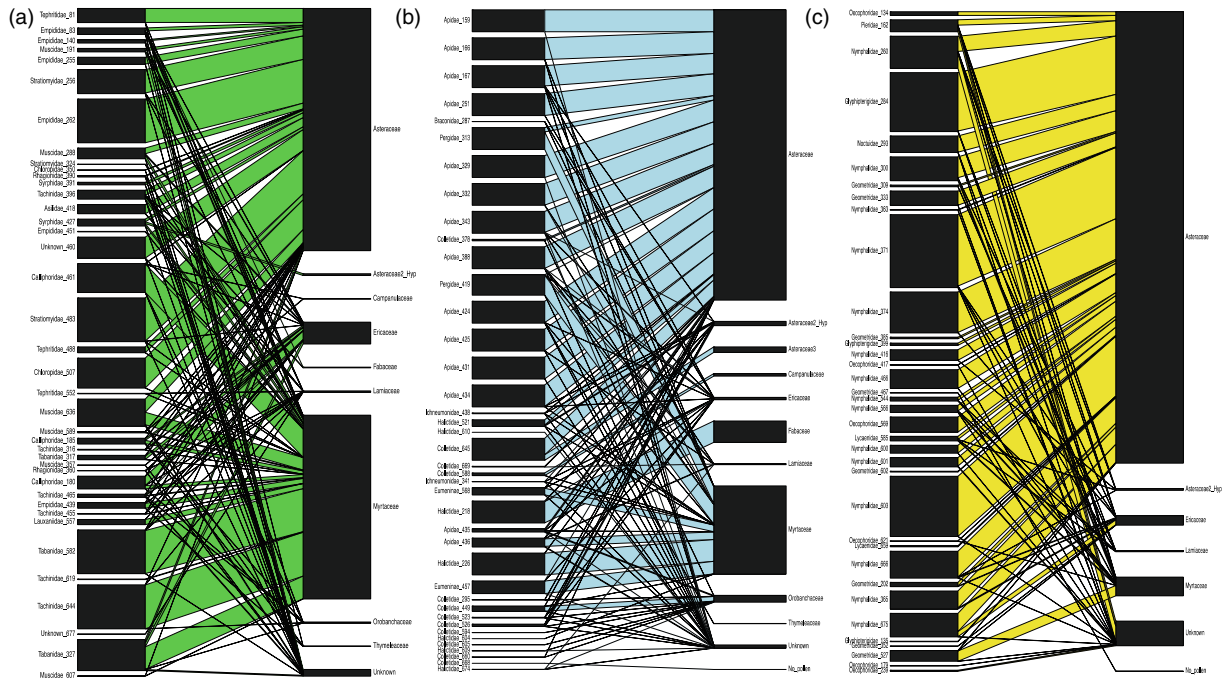


FIGURE 2 Pollen transport network separated by order (a) Diptera, (b) Hymenoptera and (c) Lepidoptera to distinguish individual-level differences across families and orders. The lines connecting the insect pollinators (right side of graph) to the plants taxa they visited (left side of graph) indicate interactions based on pollen load. Line thickness is representative of the number of pollen grains (e.g., a thicker line connecting and insect to a flower indicates a larger number of pollen grains). Coloured lines in the networks represent interactions between individual insects and plants based on pollen load. ‘Asteraceae2_Hyp’ refers to the only non-native species: *Hypochoeris radicata* L.

(Figure 2). Asteraceae had the highest NS of all plant families within the pollen transfer network (NS = 62.475), followed by Myrtaceae (NS = 26.177) and Ericaceae (NS = 8.287).

Native bee taxa carried similar amounts to the dipteran visitors (Tables S2 and S3). At the family level, Calliphoridae, Apidae and Colletidae were the families with highest NS (Table S2), whereas the taxa with the highest NS were *Senostoma rubricarinatum* (Macquart), *Calliphorid* sp. 1, *Hylaues (Prosopistemon) gracilidentatus* (Cockerell), *Lasioglossum (Austrevylaeus)* sp. (Michener) and *A. mellifera* (Table S3). Lepidoptera taxa had lower NS values than most Diptera and Hymenoptera taxa (Table S3). At the individual level, Halictidae and Colletidae carried equivalent amounts of pollen to Apidae (Figure 2). More specifically, seven individuals having the greatest importance in the network based on NS were hymenopterans (Table 1). The individual with the highest NS overall was *Lasioglossum (Austrevylaeus)* sp. Halictidae (Hymenoptera) (NS = 1.053) (‘Halictidae3’ in Figure 2 Table 1).

DISCUSSION

Bees are well known to play an important role in visiting many different species of plants and carrying large amounts of pollen (Greenleaf et al., 2007; Inouye et al., 2015; Johanson et al., 2018). In this study, bees carried more pollen overall than the other three insect orders surveyed (Kearns & Inouye, 1994), but dipterans were the most frequent flower visitors overall, with six dipteran families observed

visiting flowers more frequently than the most frequent hymenopteran visitor (*A. mellifera*). Across all taxa, flies carried more pollen from other plants than the other orders. Furthermore, the variability in the families of pollen carried by individuals was high; some individuals carried large amounts of con-familial pollen, but others carried a higher hetero-familial pollen load.

This variability among taxa has significant implications for our understanding of community-level pollen flow and confirms that identifying the most effective pollinators is complex; visitation rate by dominant taxa, irrespective of efficiency in transporting pollen, is a strong predictor of pollination success in many studies (Kleijn et al., 2015; Winfree et al., 2015). Hence, given that flies are often the dominant floral visitors in alpine environments, they could well be the most effective pollinator community in these ecosystems (Inouye & Pyke, 1988; Lefebvre et al., 2014; Lefebvre et al., 2018). Merging visitor observation and pollen transport networks can thus facilitate our understanding of community-level pollen flow and identifies missing links in both directions that would otherwise be missed (Bosch et al., 2009; Popic et al., 2013).

The main difference between flower-visitor observation and pollen transport networks is that observation networks tend to miss more plant–pollinator interactions than pollen-based networks (Bosch et al., 2009; Encinas-Viso et al., 2022), as it is shown in our study. This generates one important network structure difference between observation and pollen transport networks: pollen transport networks tend to be more generalised, that is, with higher number of interactions and lower levels of specialisation (Bosch et al., 2009; Encinas-

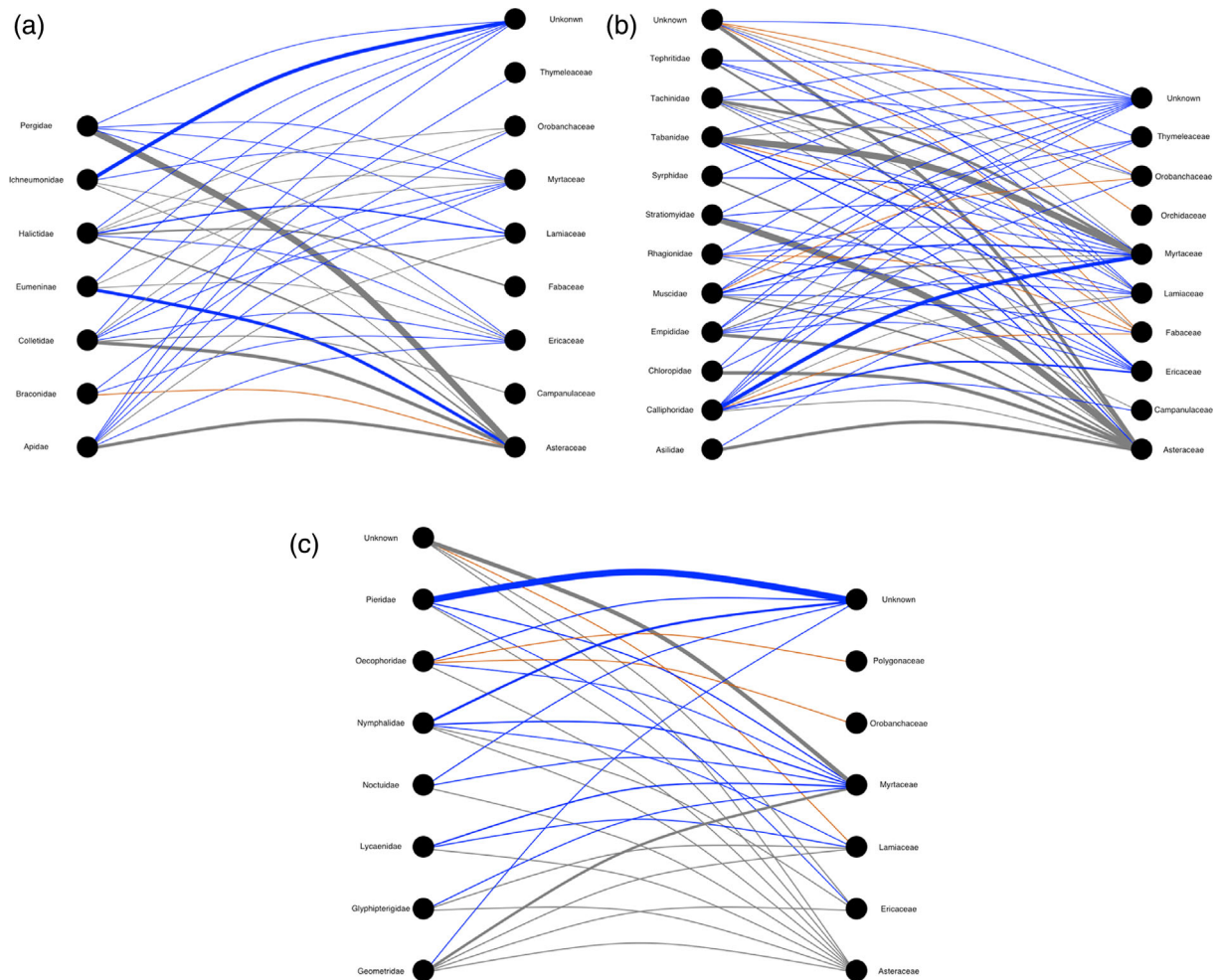


FIGURE 3 Missing links for each order (a) Hymenoptera, (b) Diptera and (c) Lepidoptera. Grey lines were links that were present in both visitor observations and the pollen transfer network, blue lines indicate links that were only present in the pollen transfer network and vermilion lines were only present in the visitor observations. Line width represents number of pollen grains. Missing links are defined as plant–pollinator interactions not recorded on the visitation network, but present on the pollen-based network.

TABLE 1 Ranking of the top 10 individuals based on node strength within the pollen transfer network.

Ranking	Ref	Insect order	Insect family	Insect species	Node strength
1.	218	Hymenoptera	Halictidae	<i>Lasioglossum (Austrevylaeus) sp.</i>	1.053
2.	645	Hymenoptera	Colletidae	<i>Leioproctus obscurus</i>	1.032
3.	588	Hymenoptera	Colletidae	<i>Hylaeus (Prosopisteron) gracilidentatus</i>	0.878
4.	449	Hymenoptera	Colletidae	<i>Hylaeus (Prosopisteron) semipersonatus</i>	0.650
5.	424	Hymenoptera	Apidae	<i>Apis mellifera</i>	0.513
6.	526	Hymenoptera	Colletidae	<i>Hylaeus (Prosopisteron) semipersonatus</i>	0.400
7.	436	Hymenoptera	Apidae	<i>Apis mellifera</i>	0.388
8.	636	Diptera	Muscidae	<i>Musca vetustissima</i>	0.375
9.	180	Diptera	Calliphoridae	cf. <i>Calliphora</i> spp.gr. 4	0.349
10.	260	Lepidoptera	Nymphalidae	<i>Oreixenica latialis</i>	0.292

Note: The three families with the highest species strength were all hymenopterans. The insect family that had the greatest NS overall was Colletidae (NS = 3.431).

Abbreviations: NS, node strength.

Viso et al., 2022). The difference is mainly explained by the difficulty of increasing sampling effort of flower-visitor observations, which is laborious and time-consuming compared with pollen-based approaches (Encinas-Viso et al., 2022; Pornon et al., 2016). Pollen-based approaches using molecular methods are becoming more accessible to infer plant–pollinator interactions and complement flower-visitor observation data (Encinas-Viso et al., 2022; Pornon et al., 2016).

Understanding the magnitude of pollen transport and the strength of interactions is a significant step forward in understanding community-wide pollen transport as it recognises both the importance of individual contributions and different combinations of frequent visitors and uncommon taxa (King et al., 2013; Ne'eman et al., 2010). NS metrics provide a valuable estimate of the relative importance of connections among both individuals and taxonomic groups within the pollen transfer network. Both bees and non-bees were ranked in the top 10 individuals that had the highest individual NS, that is, carried the greatest number of pollen grains (Table 1). Bees dominated the top seven rankings, and three non-bees made the remaining top 10 (Table 1). Although subject to sampling constraints, this study provides a method to compare the individual performance among individuals in their community.

Although we advocate that grouping taxa at the ordinal level and identifying variation among individuals are both important ways to understand community-level pollen flow, the high variability among individuals could make generalisation problematic. Moreover, variability of pollen abundance and richness among individuals can be partially explained by collecting individuals at different time points of their foraging trip. Grouping flower-visiting taxa into broad classifications—such as bees/non bees and different orders as we have done here and as practiced by a growing number of studies (Ballantyne et al., 2015; Orford et al., 2015)—could lead to misleading interpretations of interactions (Tur et al., 2014). In particular, broad groupings may overestimate or underestimate the importance of different taxa in contributing to pollination. For example, Apidae (all *A. mellifera*) was the only insect family that was recorded as having consistently high con-familial pollen loads. This is not unexpected as social species, such as *A. mellifera*, are known to have high floral constancy when foraging (Seeley, 1985). Some individuals within some dipteran families (e.g., Calliphoridae, Tabanidae and Stratiomyidae) also carried large pollen loads, but the pattern was less consistent for all individuals of that family. For example, some Calliphoridae (e.g., *Calliphora* spp.) and Tabanidae (e.g., *Copidapha maculaventris* (Westwood)) taxa from this alpine community have high hairiness and are known to carry pollen from multiple plant species (Encinas-Viso et al., 2022). This indicates that based on pollen carried, some bees (especially *A. mellifera*), some flies and some lepidopterans would likely have the greatest potential to deposit pollen effectively based on their high pollen load and relatively high proportion of con-familial grains, yet when grouped at the ordinal level were less abundant and efficient than Hymenoptera. Although we do not have yet any data regarding pollination efficiency or functional traits of Australian alpine pollinators, it seems possible that some specific traits (e.g., body size, hairiness, scopae and long proboscises) present in the pollinator community are

driving the structure and diversity of this pollen transport network (Kendall et al., 2019). For example, native bees (e.g., *Leioproctus obscurus* (Smith), *Exoneura* sp. (Cockerell)) of this community have specific traits (e.g., scopae) to carry pollen and seem to be the main pollinators of several different alpine plants (Encinas-Viso et al., 2022). Given the variation among taxa identified here and the evidence of many missing links as a result of differences in methods, merging both observational plant–pollinator network studies and pollen transfer networks captures a more complete suite of interactions in a community and enables identification of individual taxa that exhibit high NS.

A key challenge for pollination ecology is to build greater understanding of the relationship between insect visitation and pollen transfer at the community scale. Confirmation of which taxa are the most effective thus requires greater research and empirical studies that quantify pollinator importance (Inouye et al., 1994; Ne'eman et al., 2010; Willcox et al., 2017). Future studies are urgently needed to compare con-familial and hetero-familial pollen movement among individuals, space and time to understand better their relative importance among different taxa.

AUTHOR CONTRIBUTIONS

Francisco Encinas-Viso: Conceptualization; methodology; supervision; formal analysis; writing – review and editing; writing – original draft; visualization; investigation; software; data curation. **Emma Goodwin:** Conceptualization; writing – original draft; writing – review and editing; methodology; investigation; data curation; formal analysis; project administration; validation; visualization; software. **Manu E. Saunders:** Conceptualization; methodology; writing – review and editing; supervision; formal analysis; investigation. **Jaime Florez:** Methodology; data curation; writing – review and editing. **James Lumbers:** Methodology; writing – review and editing; data curation. **Romina Rader:** Conceptualization; methodology; data curation; supervision; project administration; writing – review and editing; funding acquisition; investigation; resources; formal analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The datasets generated during and analysed during the current study are available in the CSIRO Data Access Portal: <https://data.csiro.au/collection/csiro:57790v1>.

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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 insect families and morphospecies per family collected across the three insect orders.

Table S2. Node strength values of insects at the family level.

Table S3. Node strength values for all insect morphospecies.

Table S4. List of insect pollinator specimens collected and vouchered at the Australian National Insect Collection.

Figure S1. Frequency of visits for insect family observed across 3 orders (Diptera, Hymenoptera and Lepidoptera) used in this study.

Figure S2. Variation of pollen load across insect orders. Each filled circle shows values of pollen abundance from each specimen and box plots show the median (solid black line) pollen abundance per insect order. Figure produced with ‘geom_jitter’ function from R package ‘ggplot2’ in R 4.2.1 (R Core Team, 2018).

Figure S3. Pollen richness (i.e., number of species) per insect order. Each filled circle shows values of pollen richness from each specimen and box plots show the median (solid black line) pollen richness per insect order. Figure produced with ‘geom_jitter’ function from R package ‘ggplot2’ in R 4.2.1 (R Core Team, 2018).

Figure S4. Raw totals of (a) con-familial and (b) hetero-familial pollen grains for each order. Each filled circle shows values of pollen grain abundance for con-familial and hetero-familial from each specimen and box plots show the median (solid black line) pollen grain abundance per insect order. Figure produced with ‘geom_jitter’ function from R package ‘ggplot2’ in R 4.2.1 (R Core Team, 2018).

Figure S5. (a) Visitor network for the same 122 samples based on which plant family each insect order was caught visiting, and (b) Pollen transport network based on how many grains of each plant family were on the insect body. ‘Asteraceae2_Hyp’ refers to the only non-native species: *Hypochaeris radicata*.

Figure S6. Bipartite network visualisation of observed visitation, with only one interaction possible between individual insect and the plant species it was captured on. Green = Diptera, blue = Hymenoptera, yellow = Lepidoptera, grey = plant taxa.

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