



# Congruence between visitation and pollen-transport networks in a California plant–pollinator community

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Most recent studies describing pollination networks are based on observed flower visits, and few have explicitly tested if the floral visitors actually carry pollen. Since floral visitors can vary in their ability to remove and transfer pollen, it is important to show that visitation patterns reflect effective pollination. Given the difficulty of measuring per-visit pollen deposition at the community scale, a first step is to examine the amount of conspecific pollen carried by insect visitors. Here I compared the plant–animal visitation network with the pollen-transport network, estimated from insect pollen loads, for a montane meadow community from southern California, USA. Visitation and pollen-transport networks were positively associated with each other in both 2001 and 2002. However, the exclusion of visitors that do not carry any conspecific pollen reveals that pollen-transport networks are more specialized from the plants' perspective and that species are involved in fewer mutualistic interactions compared with estimates derived from visitation frequencies. Although conspecific pollen loads were smaller in 2002, bees tended to carry the largest conspecific loads in both years and were responsible for transporting the most pollen. These results suggest that, although visitation networks are suitable first-order approximations of pollination networks, information on which visitors carry conspecific pollen, and in what amounts, is crucial for distinguishing between antagonistic and mutualistic interactions.

In the late 1990s several authors questioned the idea that most plant–pollinator interactions were ecologically and evolutionarily specialized (Herrera 1996, Ollerton 1996, Waser et al. 1996, Waser 1998, Memmott 1999). The ensuing discussion has revitalized the study of entire plant–pollinator communities (Waser and Ollerton 2006), and has led to the realization that they can be viewed as complex networks that can be studied using novel analytical techniques (Proulx et al. 2005). Such studies have revealed, among other patterns that: 1) in most pollination networks the degree of generalization follows a continuous distribution, with most species having few partners and some having many, 2) that interactions tend to be asymmetric, such that specialists tend to interact with generalist partners, and 3) that pollination networks are highly nested, such that most species interact with hierarchical subsets of generalist partner species (Bascompte and Jordano 2007, Vázquez et al. 2009).

However, much of the recent progress has actually come from exploring patterns of flower visitation by animals, i.e. from visitation networks. Although visitation can provide valuable information as to the resource use and feeding association of animal visitors (Bascompte et al. 2003, Olesen et al. 2007), determining if visitors are potential pollinators requires additional effort, for example, quantification of pollen on their bodies (for exceptions see Forup and Memmott 2005, Gibson et al. 2006, Larson et al. 2006, Lopezaiza-Mikel et al. 2007, Bartomeus et al.

2008). Many workers attempt to gauge the likelihood that flower visitors are actually pollinators by recording only those visitors that actively forage for floral resources and touch the plant's reproductive structures (Memmott 1999, Alarcón et al. 2008). But flower visitors vary greatly in their ability to remove and transfer pollen. Even among those that are not obvious 'robbers' or 'cheaters', in the sense of using floral resources but transferring no pollen (Herrera 1987, Bronstein 1994), some species may transfer pollen at such a cost to plants that their effect on plant fitness is negative in the presence of better pollinators (Wilson and Thomson 1991, Lau and Galloway 2004). Thus it is conceivable that recently described pollination network properties might not truly reflect mutualistic interactions per se, because they include interactions with variable effects on plant fitness.

Given the potential problem in quantifying the fitness contribution to a plant of each flower visitor, a first approach is to remove and quantify the amount of conspecific pollen found on the bodies of visitors, so as to generate a pollen-transport network to describe how much conspecific pollen is potentially moved among flowers. One of the strengths of this approach is that it is possible to estimate the potential fitness contributions of visitors by analyzing the pollen loads of a large sample of insects in a relatively short period of time. Gaining knowledge of which visitors actually carry conspecific pollen, and in what

amounts, allows us to improve our understanding of pollination of single plant species or of groups of species, up to the level of an entire plant community. With the pollen-transport network at hand, it should be possible to assess how well observed visitation networks approximate pollination networks.

By analyzing visitation and pollen-transport patterns with the indices used to characterize ecological networks, we can also gain insight as to how the inclusion of individuals that do not carry conspecific pollen, and thus are not providing any pollination service, distorts our perception of network structure and generalization from the plants' perspective. For example, plant species may actually be more specialized, and thus more reliant on a few visitors for pollen transfer, than is suggested by visitation patterns, if only subsets of visitors actually carry conspecific pollen. The inclusion of 'cheaters' that do not pollinate could also be altering estimates of network descriptors, such as connectance and nestedness. Without identifying which visitors are potential pollinators, we will not know if the structural patterns based on visitation patterns accurately depict mutualistic networks.

Knowledge of the amounts of conspecific pollen carried by visitors also allows for comparisons across major taxonomic groups, for example the insect orders (Coleoptera, Diptera, Hymenoptera, and Lepidoptera) from which most pollinators are drawn. For example it is often asserted that bees (Hymenoptera: Apoidea) are the most important group of pollinators in natural communities (Proctor et al. 1996, Michener 2000). If this is true, bees should carry relatively pure pollen loads and transport the most conspecific pollen across the entire pollination network. If differences do exist among the various pollinator groups in the amount of conspecific pollen transferred, this would suggest that null model approaches that rely only on visitation frequencies, and ignore species identities and pollinator traits, may misrepresent the relative importance of different visitor species on plant fitness and gene flow.

Analysis of the pollen carried by insect visitors has recently been used to improve our understanding of resource use from the animals' perspective in a plant-pollinator network (Bosch et al. 2009, Discussion). However, in this paper I focus on the plants' perspective for a montane meadow community from southern California. In particular I addressed the following questions: 1) is the plant-animal visitation network, positively associated with the pollen-transport network, as estimated from the pollen carried on insect bodies? 2) Does the inclusion of flower visitors that do not carry any conspecific pollen alter our estimates of species generalization and network structure? 3) And do different taxonomic groups of insect visitors vary systematically in the amounts of conspecific pollen they carry on their bodies and transport?

## Material and methods

### Study sites and flower-pollinator surveys

Detailed descriptions of the study sites and field methods can be found in Alarcón et al. (2008). Briefly, this study was conducted in meadows located within openings in a

yellow pine (Jeffrey pine, *Pinus jeffreyi*, and ponderosa pine, *P. ponderosa*) forest in the San Bernardino Mountains of southern California (34°13'N, 116°57'W; 2250–2300 m). With the help of student interns, I surveyed each of 12 transects for pollinator visits to flowers, 1–3 times per week, for six weeks beginning in mid-June and ending in early August of 2001 and 2002. Surveys usually took place between 08:00–18:00 h under favorable weather conditions.

A pollinator survey involved a pair of observers slowly walking (20–40 min) a belt transect, and recording only those insects that contacted the plant's reproductive structures while actively searching for pollen and/or nectar. We limited our search to those insects belonging to the insect orders most commonly associated with pollination (Coleoptera, Diptera, Hymenoptera and Lepidoptera). Insects were netted and placed into individually-labeled vials, or captured directly into vials. To minimize our impact on local insect populations, only subsets of individuals from easily identifiable species, such as butterflies, were kept for subsequent pollen collection. Insects were frozen and transferred to the laboratory where they had their pollen removed. Insects were placed into operational taxonomic units, identified to the family, and higher level of resolution if possible. Visitors were also grouped into categories commonly recognized by pollination biologists: bees, beetles, butterflies, flies and wasps. Moths were excluded from all subsequent analyses because we collected too few specimens. Voucher specimens are in my possession and will be deposited at an entomological museum.

### Insect pollen loads

I removed the pollen from the body of each insect visitor by swabbing it with a small cube ( $\sim 2 \text{ mm}^3$ ) of fuchsin jelly (Kearns and Inouye 1993), making sure not to remove pollen from specialized pollen-carrying structures found on most bees, e.g. scopa, which would not be available for pollination (Michener 2000). Each fuchsin jelly cube was then placed on a glass slide, melted and covered with a cover slip to produce a single layer of stained pollen grains. Pollen slides were analyzed at 40–100 $\times$  magnification by comparison to a reference pollen library taken from plants at the study sites. I counted all the pollen grains on each slide and classified pollen as either conspecific, belonging to the plant species the insect was collected on, or heterospecific, belonging to other plant species. I did not identify heterospecific pollens to species because in most cases the numbers of heterospecific pollen grains were too few (median = 2,  $n = 1745$  slides) to unambiguously identify them.

### Statistical analysis

I pooled the observed flower-visits for a given year to create a single pollinator by plant ( $A \times P$ ) matrix, or visitation network, in which the cell values indicated the number of times individuals visitors from species 'a' were observed foraging on flowers of plant species 'p' (Alarcón et al. 2008). Similarly, for each year I constructed a pollen-load network, with the same dimensions as above, in which the cells represented the mean number of conspecific pollen

grains of plant species 'p' carried by individuals of visitor species 'a'. I also created a pollen-transport network for each year, in which cell values represent the cell-by-cell product of the visitation and pollen-load networks, i.e. the total amount of 'p' pollen transported by visitor species 'a'. In subsequent analyses I included only those species and pairwise interactions for which I was able to prepare one or more pollen slides from individuals of species 'a' foraging on flowers of plant 'p'.

**Question 1. Are plant–animal visitation networks positively associated with pollen-transport networks?**

Data from 2001 and 2002 were analyzed separately, since Alarcón et al. (2008) had shown that the visitation networks differed significantly between those years. To investigate the overall concordance among visitation, pollen-load, and pollen-transport networks, I performed orthogonal, least-squares Procrustes analyses using the FATHOM toolbox (Jones 2002) implemented in MatLab 7.1. Variables were first standardized (mean = 0, variance = 1) within the P columns so they would contribute equal weight, to allow for comparison between different types of pollination variables, e.g. visits versus conspecific pollen. The Procrustes algorithm minimizes the sums-of-squares distances between corresponding observations between two  $A \times P$  matrices by translating, reflecting, rotating and scaling one matrix to fit the other (Jackson 1995, Jones 2002). The approach then is analogous to mapping the positions of visitor 'landmarks' between superimposed pollination 'landscapes'. The resulting  $m^2$  statistic is a symmetric measure of goodness-of-fit and varies from 0 to 1, with smaller values indicating a better fit (Jackson 1995, Jones 2002). Significance of the statistic was determined by a permutation test (10 000 permutations; Jackson 1995, Peres-Neto and Jackson 2001). To make interpretation simpler, I converted  $m^2$  to a percentage of the congruence between two matrices (congruence =  $(1 - m^2) \times 100$ ).

Previous work suggests that Procrustes analysis can outperform the Mantel test in determining the concordance between matrices, and may be easier to interpret since the output also produces vector residuals for each observation, which measure the deviation in positions between the two matrices (Jackson 1995, Peres-Neto and Jackson 2001). Thus I used the vector residuals to identify the five visitor species whose estimates of pollination variables were the least concordant. For each visitor species I then calculated the mean number of visits per link, the mean number of conspecific pollen grains carried by foragers, and Sorensen's similarity index ( $SI = 2a/2a + b + c$ , where  $a$  = number of species in common,  $b$  and  $c$  = number of species unique to the visitation and pollen-transport networks, respectively) to estimate similarity in the pattern of links established. Sorensen's similarity index which ranges from 0 when no links are shared to 1 when the samples are identical.

**Question 2. Does the inclusion of flower visitors that do not carry any conspecific pollen alter our estimates of species generalization and network structure?**

To determine if the inclusion of visitors that are not carrying any conspecific pollen is altering our perception of pollination networks, I compared the structure of the

visitation and pollen-transport networks within a given year. All floral visitors observed in this study were included for visitation networks, whereas only those individuals that actually carried conspecific pollen were considered for the pollen-transport networks. For each network I then calculated the number of plant species (P), animal species (A), total number of flower visits observed (V), the number of unique plant–pollinator links recorded (K), and the proportion of possible links actually realized, or connectance ( $C = K/(A \times P)$ ). For a given year, to compare the number of links (K) recorded between visitation and pollen-transport networks as a function of the number of visits (V) recorded, I generate rarefaction curves and their 95% confidence intervals using EcoSim 7.71 (Gotelli and Entsminger 2004).

I calculated nestedness using the binary version of the  $A \times P$  networks with the ANINHADO program ([www.guimaraes.bio.br/software.html](http://www.guimaraes.bio.br/software.html)), Guimarães and Guimarães 2006). The program calculates the temperature (T) statistic, a measure of network disorder, which ranges from 0° for a perfectly nested matrix to 100° for a randomly organized matrix. The statistical significance of T is calculated by a Monte Carlo randomization procedure which generates 10 000 random matrices and their temperatures to compare with the actual matrix temperature. I used the CE null model, which limits the probability of observing an interaction between plants and pollinators as a function of their degree of specialization (Bascompte et al. 2003, Guimarães and Guimarães 2006). I followed Bascompte et al. (2003) in converting T to the nestedness index N ( $N = (100 - T)/100$ ), which ranges from 0, when the network is randomly organized, to 1, when it is perfectly nested.

I also calculated the quantitative  $H_2'$  and vulnerability indices using the bipartite package (Dormann et al. 2008) implemented in the R (ver. 2.8.1, R Development Core Team 2008) statistical package. For visitation networks, quantitative indices are based on visitation frequencies, whereas for pollen-transport networks, calculations are based on the conspecific pollen grains being transported.  $H_2'$ , the standardized Shannon diversity index, measures the degree to which the observed pattern of pairwise interactions (i.e. visits or pollen being transported) deviates from the expected pattern given the total value recorded for each species (Blüthgen et al. 2006, Dormann et al. 2009).  $H_2'$  values range from 0 to 1, with 1 representing a completely specialized network (Blüthgen et al. 2006). Vulnerability, which is also based on the Shannon diversity index, represents the weighed-mean number of visitor species (predators) per plant species (prey) of a network (Dormann et al. 2009). Thus larger vulnerability values indicate that plants species within a network are visited, or have their pollen transported, by more diverse sets of animal species.

For each network I calculated several species-level indices, including the mean number of links per plant ( $\langle k_p \rangle$ ) and animal species ( $\langle k_a \rangle$ ). I also calculated the mean standardized Kullback–Leibler distance, or specialization index ( $\langle d' \rangle$ ), to estimate plant and visitor species' relative partner diversity using the bipartite package (Dormann et al. 2008). The specialization index, which is related to  $H_2'$ , summarizes the extent to which a

species' interactions, either visits or pollen transported, deviate from the null expectation in which species interact with partners in proportion to their abundance (Blüthgen et al. 2006). The specialization index varies from 0 to 1, with 1 representing highly specialized partner use (Blüthgen et al. 2006).

Lastly, I calculated the mean interaction strength of animal visitors ( $<IS_a>$ ). For visitation networks, the strength exerted by visitor 'a' on plant 'p' was calculated as the proportion of visits between 'a' and 'p', relative to p's total visits (Vázquez et al. 2007). I then averaged these values across all the plant species that visitor 'a' interacted with. For pollen-transport networks, the strength exerted by visitor 'a' on plant 'p', was calculated as the proportion of 'p' conspecific pollen carried by 'a', relative to all of p's conspecific pollen carried by visitors. I then averaged these values across all the plant species that visitor 'a' carried conspecific pollen for. To compare mean interaction strength, and the other species-level index values, between visitation and pollen-transport networks, I performed one-tailed, paired t-tests. Only those species present in both the visitation and pollen-transport networks were included in these comparisons.

### **Question 3. Do different taxonomic groups of insect visitors vary systematically in the amounts of conspecific pollen they carry on their bodies and transport?**

To evaluate whether different taxonomic groups (bees, beetles, butterflies, flies and wasps) varied in the composition of their pollen loads, or whether there is annual variation in insect pollen loads, I performed two-way ANOVAs, followed by post-hoc Tukey HSD tests to correct for multiple comparisons. Specifically, I compared the number of conspecific pollen grains and the proportion of conspecific pollen carried on insect bodies. To meet the assumptions of homogeneity of variance and normality, numbers of conspecific pollen grains carried were log-transformed ( $\log_{10}(X+1)$ ), whereas proportions of conspecific pollen carried were arcsine-transformed ( $\arcsin(\sqrt{p})$ ); but I report back-transformed values.

Since visitor species can vary in how much conspecific pollen they move within a network because of differences in individual pollen load size and/or abundance, I also estimated the total amount of conspecific pollen being transported by taxonomic groups. To arrive at group-level pollen-transport values, I summed species-level pollen-transport estimates, which were calculated as the mean number of conspecific pollen grains per individual, multiplied by the number of foragers observed for a given visitor species. I then used G-tests to test if the groups differed in the amounts of conspecific pollen they are transporting.

## **Results**

The 2001 visitation network had dimensions of  $121 \times 31$  ( $A \times P$ ), based on 949 flower-visits ( $V$ ) recorded that year (see Alarcón et al. 2008 for details on entire visitation networks). The pollen-load and pollen-transport networks had equivalent dimensions as the visitation network, and were based on pollen slides from the bodies of 759 insect

specimens, for a combined total of 101 793 pollen grains counted. The 2002 visitation network had dimensions of  $118 \times 38$  ( $A \times P$ ), based on 1147 flower-visits ( $V$ ) observed that year. The corresponding 2002 pollen and pollen-transport matrix were based on pollen slides from the bodies of 986 insect specimens and a combined total of 217 585 pollen grains counted. Of the insects collected, a significantly greater proportion of them carried no pollen at all in 2002 (19.6%), compared to 2001 (8.6%; G-test,  $G_1 = 32.70$ ,  $p < 0.001$ ).

### **Question 1. Are plant-animal visitation networks positively associated with pollen-transport networks?**

Procrustes analyses of visitation, pollen-load and pollen-transport networks suggest that they were positively associated with each other in both 2001 and 2002. In 2001, the visitation network (based on the number of foragers from species 'a' observed visiting flowers of 'p') showed a moderate match to the pollen-load network (mean number of 'p' conspecific pollen grains carried by 'a' foragers), with 41.27% congruence (critical  $\alpha = 0.0167$ ;  $m^2 = 0.5873$ ,  $p = 0.0001$ ). The visitation network showed an even stronger match to the pollen-transport network (estimated total amount of 'p' conspecific pollen transported by 'a'), with a 63.08% congruence ( $m^2 = 0.3692$ ,  $p = 0.0001$ ). However, the pollen-transport network showed the greatest resemblance to the pollen-load network with an 80.17% congruence ( $m^2 = 0.1983$ ,  $p = 0.0001$ ). Similar patterns of network concordance were detected in 2002 with the visitation network exhibiting a stronger resemblance to the pollen-transport network, with 63.50% congruence ( $m^2 = 0.3650$ ,  $p = 0.0001$ ), compared to the pollen-load network, at 53.74% congruence ( $m^2 = 0.4626$ ,  $p = 0.0001$ ). As before, the 2002 pollen-transport network exhibited the greatest resemblance to the pollen-load network, 84.43% congruence ( $m^2 = 0.1557$ ,  $p = 0.0001$ ). These results suggest that conspecific pollen loads are correlated with visitation patterns and that the total amount of conspecific pollen being transported by a visitor species is related both to individual pollen load size and visitation patterns, but more strongly to the former.

For brevity, I only present the residual vectors from the Procrustes analysis for the five visitor species with the greatest deviations between visitation and pollen-load networks (Table 1), since several of the visitor species were among the species with the largest residuals from the other analyses. In some cases it appears that species had foragers that carried large quantities of conspecific pollen, but were rather infrequent visitors (Table 1). For example, in 2002 foragers of the leaf-cutter bee, *Megachile* sp. 1 (Megachilidae), were observed rather infrequently on the flowers of the plant species that they interacted with (3.5 visit per link), yet they carried very large amounts of conspecific pollen (mean = 805.9 grains). On the other hand, the small flies of Milichiidae sp. 1 (Diptera), were commonly observed on the flowers of the plants that they interacted with (12.8 visits per link), but carried very little conspecific pollen (mean = 0.6 grains). And some visitors were both rare and carried little pollen: for example the hoverfly, *Platycheirus obscurus* (Syrphidae), was observed infrequently (1.2 visits per link) in 2001, and carried comparably small

Table 1. Residual vectors from Procrustes analyses for the top five visitor species with the greatest deviations between visitation and pollen-load networks, along with the total number of foragers observed (n), mean number of visits per link, the mean number of conspecific pollen grains carried per forager, and Sorensen's similarity index (SI) for links established (ranges =0–1, with 1 representing identical samples).

Species	Order	Residuals	n	Visits per link	Conspecific pollen load	SI
2001						
Tenthredinidae sp. 1	Hymenoptera	0.2404	3	1.5	1515.7	1.00
Melyridae sp. 5	Coleoptera	0.2401	103	10.3	110.4	0.95
<i>Platycheirus obscurus</i>	Diptera	0.2207	6	1.2	0.3	0.33
<i>Apis mellifera</i>	Hymenoptera	0.2050	124	20.7	140.9	0.80
<i>Coelioxys</i> sp. 1	Hymenoptera	0.1682	1	1.0	391.0	1.00
2002						
<i>Apis mellifera</i>	Hymenoptera	0.1786	9	4.5	538.6	1.00
Milichiidae sp. 1	Diptera	0.1689	64	12.8	0.6	0.75
<i>Megachile</i> sp. 1	Hymenoptera	0.1646	38	3.5	805.9	0.95
<i>Anthophora urbana</i>	Hymenoptera	0.1574	3	1.0	18.3	0.80
<i>Melissodes</i> sp. 1	Hymenoptera	0.1558	2	2.0	9000.0	1.00

pollen loads (mean = 0.3 grains), but only shared 33% of its links between visitation and pollen-transport networks, because only one of its foragers carried any conspecific pollen. Overall, inspection of residual vectors suggests that even though visitation and pollen-load networks are positively associated with each other, individual visitor species varied in their degree of concordance.

**Question 2. Does the inclusion of flower visitors that do not carry any conspecific pollen alter our estimates of species generalization and network structure?**

When individuals that carried no conspecific pollen were excluded, fewer visits (V), plants (P), and animal species (A) were recorded from the pollen-transport networks compared with visitation networks in both 2001 and in 2002 (Table 2). Rarefaction curves suggest that when the number of visits (V) is accounted for, significantly fewer plant–pollinator links (K) were established in pollen-transport networks compared with visitation networks (Fig. 1).

The differences in connectance (C) values, which are based on presence–absence data, were minimal between visitation and pollen-transfer networks, increasing by 6.7% in 2001 and 1.5% in 2002 (Table 2). Similarly, nestedness (N) values remained relatively unchanged between visitation and pollen-transport networks (2001 = 2.3% decrease, 2002 = 2.0%). However  $H_2'$  and vulnerability values, which are based on quantitative data, changed considerably. The  $H_2'$  index, which is a network-level estimate of specialization, increased by ~94% when comparing the visitation to the pollen-transport network in 2001, and by 35% in 2002 (Table 2). On the other hand vulnerability values, or the weighed-mean diversity of visitor species per plant species, decreased by 60% when comparing the visitation to the pollen-transport network in 2001 and by 46% in 2002 (Table 2).

Species-level indices also differed between visitation and pollen-transport networks. In both years, plants exhibited significantly fewer links per species  $\langle k_p \rangle$  in pollen-transport networks, compared with visitation

Table 2. Network and mean species-level index values for visitation and pollen-transport networks. Visitation networks included all floral visitors, whereas pollen-transport networks only included those individuals that carried conspecific pollen on their bodies. Species-level index values were compared using one-tailed paired t-tests.

	2001			2002		
	Visitation network	Pollen transport network	Significance	Visitation network	Pollen transport network	Significance
Plants (P)	31	27		38	33	
Animals (A)	121	92		118	103	
Links (K)	279	197		291	225	
Visits (V)	949	788		1147	1046	
Connectance (C)	0.074	0.079		0.065	0.066	
Nestedness (N)	0.946	0.924		0.948	0.929	
$H_2'$	0.416	0.806		0.551	0.742	
Vulnerability	12.655	4.958		8.134	4.366	
links per animal species $\langle k_a \rangle$	2.306	1.628	***	2.466	1.906	***
links per plant species $\langle k_p \rangle$	9.000	6.255	***	7.657	5.921	***
animal specialization index $\langle d'_a \rangle$	0.200	0.397	***	0.152	0.429	***
plant specialization index $\langle d'_p \rangle$	0.315	0.655	***	0.268	0.641	***
animal interaction strength $\langle IS_a \rangle$	0.080	0.123	*	0.078	0.102	†

\*\*\*  $p < 0.001$

\*  $p < 0.05$

†  $p < 0.10$

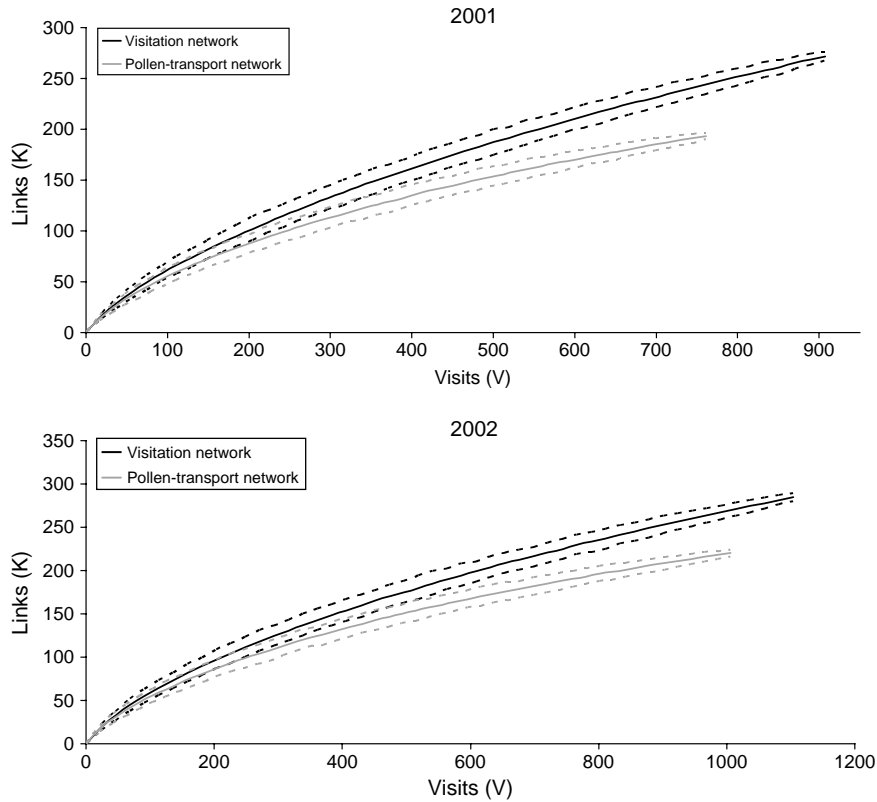


Figure 1. Rarefaction curves ( $\pm 95\%$  CI) for the number of unique plant-visitor links (K) recorded versus the number of visits (V) observed for visitation and pollen-transfer networks. The exclusion of foragers that do not carry conspecific pollen in pollen-transfer networks results in fewer links per visits recorded.

networks (Table 2). Similarly, visitor species exhibited fewer links per species  $\langle k_a \rangle$  in pollen-transport networks relative to visitation networks. Plant and animal species also had significantly greater specialization index values,  $\langle d' \rangle$ , in pollen-transport networks compared with their values in visitation networks (Table 2). Estimates of visitor interaction strength  $\langle IS_a \rangle$  significantly increased in 2001 when conspecific pollen loads were considered. However  $\langle IS_a \rangle$  values were only marginally greater from the pollen-transport network compared to the visitation network in 2002 (Table 2).

**Question 3. Do different taxonomic groups of insect visitors vary systematically in the amounts of conspecific pollen they carry on their bodies and transport?**

In 2001 I observed differences in the number of species per taxonomic group (G-test,  $G_4 = 61.4$ ,  $p < 0.001$ ), with more

fly ( $n = 56$ ) and bee species ( $n = 31$ ), than beetle ( $n = 14$ ), wasp ( $n = 14$ ) or butterfly species ( $n = 6$ ). Similarly, in 2002 there were significant differences in the number of species per group ( $G_4 = 44.0$ ,  $p < 0.001$ ), with more bee ( $n = 45$ ) and fly species ( $n = 32$ ) compared with wasp ( $n = 22$ ), beetle ( $n = 14$ ) and butterfly species ( $n = 5$ ). The overall distribution of the number of individuals per taxonomic groups also differed between years (G-test,  $G_4 = 214.3$ ,  $p < 0.001$ ; Fig. 3), but in general I observed more bees ( $n = 802$ ), beetles ( $n = 519$ ), and flies ( $n = 468$ ), than wasps ( $n = 176$ ) or butterflies ( $n = 131$ ) over both years.

Insect visitors tended to carry more conspecific pollen on their bodies in 2001 compared to 2002 ( $F_{1,1745} = 3.832$ ,  $p = 0.050$ ; Table 3). Wasp and bee foragers carried the most conspecific pollen grains, followed by beetles, with flies and butterflies carrying the fewest ( $F_{4,1745} =$

Table 3. Back-transformed mean ( $\pm 95\%$  CI) conspecific pollen load values for taxonomic groups and years. Groups not sharing the same letter are statistically different.

	Mean number of conspecific pollen grains (95% CI)			Mean proportion of conspecific pollen (95% CI)		
2001	9.8	(7.3–12.9)	a	0.488	(0.418–0.557)	a
2002	6.8	(5.4–8.5)	b	0.414	(0.361–0.469)	a
Bees	22.1	(17.8–27.3)	a	0.549	(0.491–0.605)	a
Beetles	7.9	(6.3–9.7)	b	0.510	(0.458–0.562)	a
Butterflies	1.5	(0.4–3.7)	c	0.305	(0.162–0.472)	ab
Flies	3.3	(2.5–4.3)	c	0.312	(0.262–0.366)	b
Wasps	28.2	(19.0–41.4)	a	0.587	(0.484–0.687)	a

45.243,  $p < 0.001$ ; Table 3). Most taxa carried larger numbers of conspecific pollen grains in 2001 than 2002, but the reverse was true for butterflies (Fig. 2a); however there was no significant year by taxon interaction ( $F_{4,1745} = 2.121$ ,  $p = 0.076$ ).

There was no significant difference in the mean proportion of conspecific pollen carried by insect visitors in 2001 compared with 2002 ( $F_{1,1745} = 2.609$ ,  $p = 0.106$ ; Table 3). However, bees, wasps, and beetles tended to carry proportionally more conspecific pollen than flies and butterflies ( $F_{4,1745} = 12.461$ ,  $p < 0.001$ ; Table 3). Most taxa tended to carry greater proportions of conspecific pollen in 2001 than 2002, but the reverse was true for butterflies (Fig. 2b), giving rise to a significant year by taxon interaction ( $F_{4,1745} = 3.898$ ,  $p = 0.004$ ).

There were significant differences in the total amount of conspecific pollen being transported across taxonomic groups in 2001 ( $G_4 = 109113.1$ ,  $p < 0.001$ ) and in 2002 ( $G_4 = 318048.7$ ,  $p < 0.001$ ). Bees transported the most conspecific pollen, carrying 64% and 78% of all pollen transported in 2001 and 2002, respectively (Fig. 3). Beetles transported 16.8% of all conspecific pollen in 2001 and 5% in 2002, whereas wasps transported 9.7% in 2001 and 14.4% in 2002. Although flies were well represented, they only transported 9.7% of the conspecific pollen in 2001 and 1.9% in 2002, while butterflies transported less than 1% in both years (Fig. 3).

## Discussion

The analyses presented here suggest that visitation of flowers by insects is indeed correlated with the amount of conspecific pollen carried on their bodies, and the amounts of conspecific pollen being transported per visitor species, at the level of an entire community. Congruence between visitation and pollen-transport networks was observed in both 2001 and in 2002, even though many aspects of the plant-pollinator network in this California community differed significantly between these years (Alarcón et al. 2008). Given the scarcity of community-level analyses of this kind (but see Bosch et al. 2009), the results are noteworthy and indicate the value of analyzing individual pollen loads and using matrix comparison methods, such as Procrustes analysis, to compare aspects of pollinator effectiveness to better understand the potential fitness effects of interactions from the plants' perspective.

Although visitation networks seem to be a suitable first-order approximation for the more difficult to obtain pollination networks, i.e. pollinator contributions to the reproductive component of plant fitness, they actually include interactions with variable effects on plant fitness. For example, many visitors included in the visitation networks did not carry any conspecific pollen, and thus are not potential pollinators. In fact, 25% and 34% of the insects sampled did not carry conspecific pollen on their

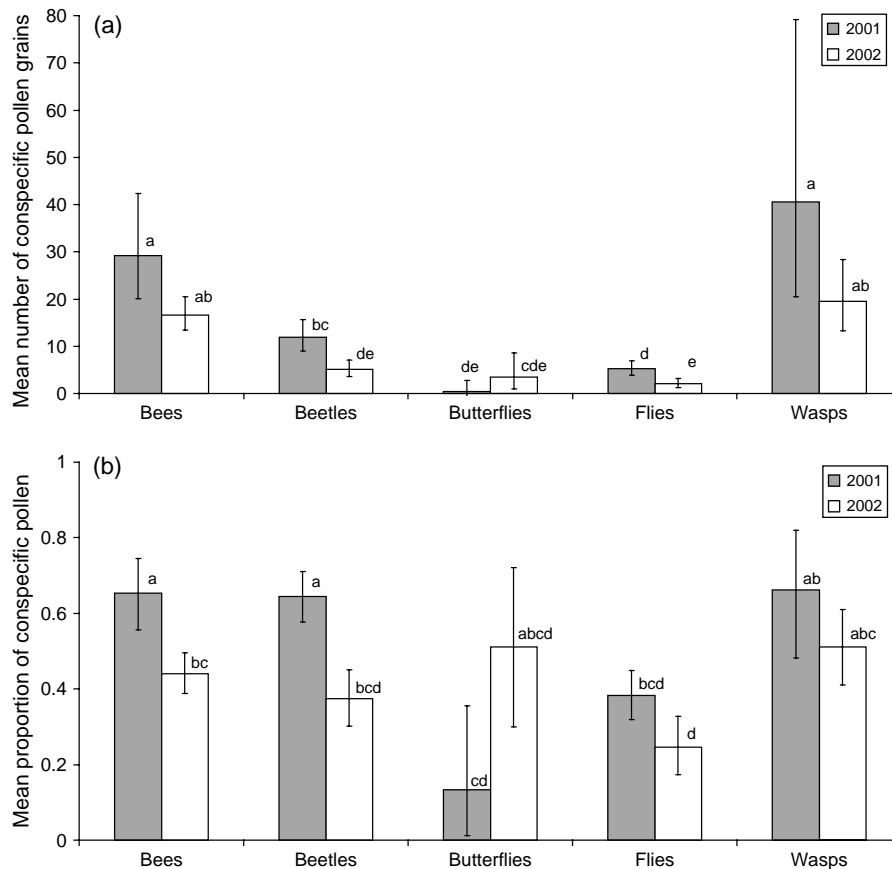


Figure 2. Least square mean ( $\pm 95\%$  CI) number of conspecific pollen grains (a) and proportion of conspecific pollen (b) carried by taxonomic groups in 2001 and 2002. Groups not connected by the same letter are statistically different.

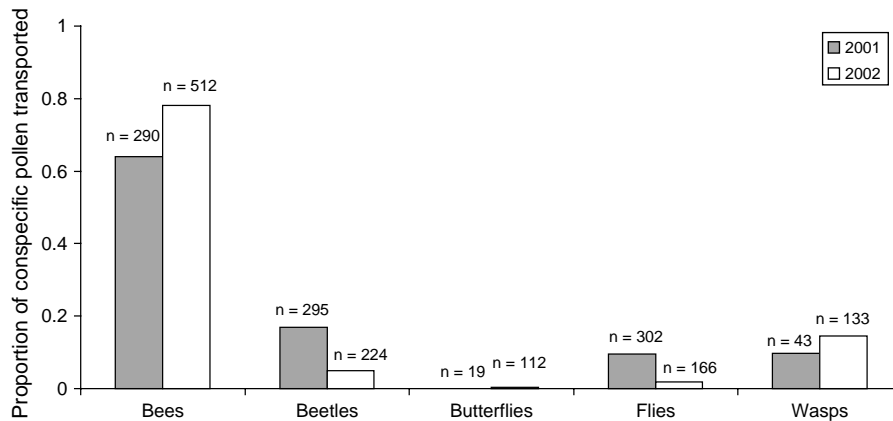


Figure 3. Proportion of total conspecific pollen being transported by taxonomic groups in 2001 and 2002. Values above bars indicate the number of individuals per group observed that year.

bodies in 2001 and 2002, respectively. Thus a substantial proportion of visitors observed in any given year are likely to be ‘cheaters’ from the plants’ perspective, in the sense of obtaining floral resources but not transferring any pollen (Bronstein 1994). Since pollen-load networks contain information as to which visitors actually carry conspecific pollen, and in what amounts, they are better approximations of pollination networks. Pollen-transport networks represent even higher-order approximation, since they incorporate estimates of both quantity (i.e., number of visits) and quality (i.e. number of conspecific pollen grains) components of pollination (Herrera 1987, 1989; for other aspects of quantity and quality see Mitchell et al. 2009), and thus characterize the potential fitness contributions, or importance (Gibson et al. 2006) of visitor species for an entire plant community (Vázquez et al. 2005, Sahli and Conner 2006).

If pollen-transport networks represent better approximations of pollination networks from the plants’ perspective, then the inclusion of individuals that do not carry conspecific pollen in the analysis of visitation networks is altering our perception of pollination network structure. For example, pollen-transport networks are smaller, in terms of the number of species, visits and pair-wise links recorded, compared to visitation networks. Furthermore, pollen-transport networks are more specialized. Thus analysis of pollen-transport networks suggests that plants actually interacted with fewer potential pollinators and animal species than what visitation patterns would have us believe (Ollerton 1996, Bischoff 2008). Not surprisingly, however, connectance (C) values did not respond very much to the structural differences between visitation and pollen-transport networks, given that connectance is based on presence absence data and exhibits a narrow range of values (Jordano 1987, Blüthgen et al. 2006). Similarly, estimates of nestedness did not differ very much between visitation and pollen-transport networks, which might related to the fact that nestedness is not sensitive to sampling biases (Nielsen and Bascompte 2007). Thus it seems that qualitative visitation and pollen-load data do not always provide sufficient resolution to detect differences in species interactions and network structure (Blüthgen et al. 2006, Alarcón et al. 2008).

This study is not unique in its analysis of visitation and insect pollen loads at the community level. Recently, Bosch et al. (2009) characterized a Spanish scrubland community from the animals’ perspective. They analyzed the pollen carried by 495 individuals and found that when pollen-load data were combined with field observations, their network contained more links compared with estimates based on visitation patterns alone, suggesting that the inclusion of pollen-load data provides more detailed information about resource use by visitors. However, these authors did not distinguish between visitors that did and did not carry conspecific pollen. Thus they overestimate the degree of generalization from the plants’ perspective by including cheaters, just as the approach I present underestimates the degree of generalization from the animals’ perspective by not identifying heterospecific pollen to species. Other recent studies analyzed insect pollen loads from an applied perspective to determine if plant–pollinator interactions had been restored to British hay meadows (Forup and Memmott 2005), and to evaluate the degree to which invasive (Larson et al. 2006, Lopezaraiza-Mikel et al. 2007, Bartomeus et al. 2008) and rare plant species (Gibson et al. 2006) were integrated into their respective communities. In fact, Larson et al. (2006) and Bartomeus et al. (2008) quantified pollen deposited on stigmas, but did so on a per-plot rather than on per-visit basis. The ideal resolution would be to measure pollen transfer by each visitor, but the logistical difficulty of doing so is enormous. Nonetheless, these studies demonstrate the importance of incorporating pollen analyses with network-level approaches to improve our understanding of plant–pollinator interactions from both the plants’ and animals’ perspective.

In addition to revealing important differences in network structure from the plants’ perspective, my analysis also showed that taxonomic groups differed in the absolute and relative amounts of conspecific pollen that they carried on their bodies. Bees and wasps tended to carry more conspecific pollen than other insects, which is consistent with some previous reports (Herrera 1987, Bartomeus et al. 2008). This might be expected given that bees, and some wasps, actively forage for pollen, have hairy bodies, and have evolved specialized structures for carrying pollen (Michener 2000). Interestingly, beetles, which are



not regarded to be very important pollinators in temperate zones (Proctor et al. 1996), also tended to carry proportionately more conspecific pollen, comparable to that of bees, which again agrees with some previous reports (Bartomeus et al. 2008), but not others (Herrera 1987). However, bees transported the most conspecific pollen overall in both years, which is similar to other meadow systems (Forup and Memmott 2005). Furthermore, taxonomic differences in conspecific pollen loads and transport suggest that null model approaches that rely only on visitation patterns are misrepresenting the relative importance of different visitor taxa on plant fitness, since differences in foraging behavior, body size, hairiness, etc., can influence their ability to pick up, carry, and deposit conspecific pollen (Herrera 1987, Fishbein and Venable 1996, Gómez and Zamora 1999, Young et al. 2007). However, coarse taxonomic affinities should not be used without additional evidence to gauge pollinator effectiveness, since species from different taxonomic groups can be functionally equivalent in transferring pollen (Gómez and Zamora 1999), while species within the same taxonomic group can vary substantially (Wilson and Thomson 1991, Adler and Irwin 2006).

Although the general patterns of pollen load composition were somewhat similar in both years, on average insects carried more conspecific pollen in 2001 than in 2002. A significantly greater proportion of the insects carried no pollen at all in 2002, which happened to be a severe drought year (Alarcón et al. 2008). The fact that such a large fraction of the specimens in 2002 carried no pollen suggests that pollen from flowers was being depleted more quickly in that year. This agrees with Alarcón et al.'s (2008) observation that fewer flowers were produced and that more insect visitors functioned as generalists in 2002 than in 2001. Thus, in addition to altering visitation patterns (Alarcón et al. 2008) drought conditions could impact pollination by reducing the number of flowers produced and pollen available for transport.

Overall, this study strongly suggests that although visitation networks are suitable first approximations for pollination networks, they include interactions with variable effects on plant fitness. Thus information on which visitors carry conspecific pollen, and in what amounts, is crucial for distinguishing between antagonistic and mutualistic interactions. As such, the inclusion of cheaters in visitation networks inflates estimates of generalization from the plants' perspective, making it difficult to determine which taxa are influencing plant fitness. Indeed, much additional work is needed on how traits such as foraging behavior (Young et al. 2007) and morphology (Stang et al. 2006, Vázquez et al. 2009) influence pollen transfer in generalized pollination systems, if we are to understand floral evolution within a community context.

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