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ORIGINAL ARTICLE

Pollen analysis reveals the effects of uncovered interactions, pollen-carrying structures, and pollinator sex on the structure of wild bee–plant networks

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Abstract Pollination networks are increasingly used to model the complexity of interactions between pollinators and flowering plants in communities. Different methods exist to sample these interactions, with direct observations of plant–pollinator contacts in the field being by far the most common. Although the identification of pollen carried by pollinators allows uncovering interactions and increasing sample sizes, the methods used to build pollen-transport networks are variable and their effect on network structure remains unclear. To understand how interaction sampling influences the structure of networks, we analyzed the pollen found on wild bees from eight communities across Mallorca Island and investigated the differences in pollen loads between bee body parts (scopa vs. body) and sexes. We then assessed how these differences, as well as the uncovered interactions not detected in the field, influenced the structure of wild bee–plant networks. We identified a higher quantity and diversity of pollen in the scopa than in the rest of the female body, but these differences did not lead to differences in structure between plant-pollination (excluding scopa pollen) and bee-feeding interaction (including scopa pollen) networks. However, networks built with pollen data were richer in plant species and interactions and showed lower modularity and specialization (H_2'), and higher nestedness than visitation networks based on field observations. Female interactions with plants were stronger compared to those of males, although not richer. Accordingly, females were more generalist (low d') and tended to be more central in interaction networks, indicating their more key role structuring pollination networks in comparison to males. Our study highlights the importance of palynological data to increase the resolution of networks, as well as to understand important ecological questions such as the differences between plant-pollination and bee-feeding interaction networks, and the role of sexes in pollination.

Key words palynology; pollen-transport networks; pollination; role of bee sexes; sampling methodology; visitation networks

Introduction

The process of pollination is a crucial ecosystem service supplying many of our resources (Gallai *et al.*, 2009). It is estimated that up to 87.5% of angiosperms (308 006 species) and 70% of the main crop species used in the world depend on pollinators for their reproduction

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(Williams, 1994; Klein *et al.*, 2007; Gallai *et al.*, 2009; Ollerton *et al.*, 2011; Potts *et al.*, 2016). In the last decades, there has been a growing interest toward the study of plant–pollinator interactions at the scale of communities, revealing complex and multispecific organizational phenomena that are relevant to the understanding of the entire ecosystem (Memmott *et al.*, 2004; Bascompte & Jordano, 2007). To study these interaction complexes, researchers have used pollination networks, which model the extreme complexity of pollination interactions in an ecosystem (Jordano *et al.*, 2006; Newman, 2018; Vizentin-Bugoni *et al.*, 2018). The use of pollination networks addressing community scale interaction patterns allowed to define crucial community parameters such as robustness to disturbance (Kaiser-Bunbury *et al.*, 2010; Thébault & Fontaine, 2010), habitat restoration and community conservation (Kaiser-Bunbury & Blüthgen, 2015; Kaiser-Bunbury *et al.*, 2017) or the impact of invasive species (Bartomeus *et al.*, 2008; Parra-Tabla & Arceo-Gómez, 2021). Understanding these networks and the factors impacting the conclusions drawn from them is therefore of primary importance to effectively address ecological and evolutionary questions at the community level.

However, the methods used to detect pollination interactions vary greatly between studies (Vizentin-Bugoni *et al.*, 2018). Traditional pollination networks, called "visitation networks," are constructed by observing direct insect visitation to flowers in the field (Vizentin-Bugoni *et al.*, 2018). While visitation networks are by far the most common in literature, other scientists have opted for the identification of pollen grains (Palynology) transported by pollinators. This less prevalent technique has been used to build "pollen-transport networks" based on pollen data, or to complement field observations leading to "mixed networks" that include the information of both visitation and pollen-transport networks (Bosch *et al.*, 2009; Jordano *et al.*, 2009; Freitas *et al.*, 2014; Jordano, 2016; Souza *et al.*, 2021). The use of palynological data has numerous advantages (Bosch *et al.*, 2009; Vizentin-Bugoni *et al.*, 2018). First, the pollen loads present on pollinators are short-term markers of interactions and, therefore, allow sampling interactions from a wider temporal scale compared to single field observations. Second, pollen analysis allows determining the efficiency of pollinators after floral visits and to detect interactions that do not result in pollen transfer (e.g., nectar robbing, Genini *et al.*, 2010). Third, palynological studies allow the study of interaction diversity in rare pollinators. In any case, the methodology used to sample interactions may affect the resulting network features and, therefore, the ecological conclusions drawn from them.

Although some recent studies have directly or indirectly compared sampling methodologies with the aim of understanding their effect on network structure (Bosch *et al.*, 2009; Alarcón, 2010; Vianna *et al.*, 2014; Souza *et al.*, 2021), the results are not always consistent. In addition, different works build pollen-transport networks in diverse ways, as for instance including (Bosch *et al.*, 2009; Vianna *et al.*, 2014) or excluding (Alarcón, 2010; Souza *et al.*, 2021) the pollen loads present in the scopa, which makes difficult to extract clear patterns from these studies. Indeed, it is a common practice to exclude the pollen loads that the female of most bee species bear in specialized structures (i.e., scopa) to best reflect pollen transfer between plants (Alarcón, 2010; Zhao *et al.*, 2019; Souza *et al.*, 2021), because this pollen might be unavailable for plant pollination (Michener, 2000). However, from the pollinator point of view, the pollen carried in the scopa may show feeding interactions that remain hidden when the scopa pollen is excluded. Therefore, the comparison of pollen loads both in the specialized carrying structures and the rest of the body is needed to understand the differences in the structure of plant-pollination interactions versus bee-feeding interactions, as well as to quantify the potential bias in network studies related to including or excluding pollen-carrying structures from pollen analysis. Such a comparison would also allow to clarify important phenomena, such as the efficiency of pollen transfer (Parker *et al.*, 2015). A lower abundance and diversity of pollen grains on the body compared to the scopa can be expected, since pollen may remain longer in the specialized structures. However, we are not aware of any study that has performed such a comparison so far.

Pollen analysis also allows studying the role of network entities at different levels, from species to individuals (Tur *et al.*, 2014; Valverde *et al.*, 2016), and therefore, it also permits assessing the role of sexes in pollination. Bees need two food sources: nectar and pollen. While both sexes obtain their resources from interacting with flowers, male and female bees do not interact with plants equally. Whereas nectar is used by all adults, pollen is only used as a source of proteins for larval growth and, as a consequence, it is exclusively collected by females, which are morphologically adapted to this task using their scopa as a specialized carrying structure (Willmer & Stone, 2004; Danforth *et al.*, 2019). Moreover, it has been shown that the two sexes of the same species can have strongly different food niches and interact with different flowering species (Roswell *et al.*, 2019). This can lead to intraspecific variation in the interactions of a similar magnitude than interspecific variations (Roswell *et al.*, 2019). Due to these morphological and ethological differences, it is expected that bee sexes differ in their role in networks, with females showing higher interaction diversity, pollination efficiency, and importance structuring the networks. Although there is evidence for the importance of both sexes in pollination (Ogilvie & Thomsom, 2015), this topic is still barely known (but see Cullen *et al.*, 2021) and more studies are needed to evaluate the differences between sexes in the quantity and richness of pollen transported, and in their role in pollination networks.

In this study, we used data on wild bee–plant visitation and pollen-transport networks from eight wild *Olea europaea* communities of Mallorca Island (Western Mediterranean, Spain) to understand how bee-carrying structures and sexes, as well as the methodology used to sample interactions impact the structure of interaction networks. We focused on bees because they are considered as the main pollinator group, since they are evolutionarily adapted for pollen transport (Michener, 2000; Mayfield *et al.*, 2001) and have interacted closely with flowering plants since the Cretaceous period (Poinar & Danforth, 2006; Hu *et al.*, 2008; Souza *et al.*, 2021). Particularly, we asked: (1) Is there a difference between the pollen found on the specialized carrying structures of the apoids (scopa) and the pollen carried on the rest of their body in terms of pollen transfer and interaction strength and richness? (2) Do the two apoid sexes differ in their ability to transport pollen after a floral visit, and in the strength and richness of associated interactions? (3) How does the method used to sample interactions (visitation networks, pollen-transport networks including and excluding scopa, mixed networks) affect the overall structure of pollination networks? (4) Do the two apoid sexes differ in their specialization and position in the interaction networks?

Materials and methods

Study communities

We selected for this study eight wild communities across Mallorca Island, in the Balearic Islands Archipelago, Spain (Fig. S1). These natural communities were all shrubland with typical Mediterranean vegetation dominated by wild *Olea europaea*. This type of communities is rich in plant species with an average (± SE) of 120.85 ± 4.37 species (range: 75–156) and 36.50 ± 0.92 families (range: 30–45) per study site (Cursach *et al.*, 2020). Study communities were ≥ 1 hectare in size, were located far from urban areas, and separated at least by 2.5 km with a mean distance of 35.57 ± 3.25 km between them. More details about the study area and the study communities can be found in Cursach *et al.* (2020), Gómez-Martínez *et al.* (2022), Lázaro *et al.* (2022), and Lázaro and Gómez-Martínez (2022).

Field sampling

In 2018, three researchers recorded wild bee–plant interactions in the field by walking slowly for 1 h along three permanent transects of 100 m × 2 m located within each study community (two in the extremes and one in the center of the study hectare). We carried out seven bee surveys at each community over a period from spring (five times) to autumn (two times), which correspond to the main flowering peaks in Mallorca. Surveys were conducted between 9:30 am and 4:30 pm, in sunny and windless days. We captured the bees when observed them contacting the reproductive parts of a flower, using a hand-net, and stopping the watch during insect manipulations. We then isolated them in new, clean Eppendorf tubes immediately after their capture to avoid pollen contamination between individuals, as well as to limit bee movements and prevent cleaning behavior that may lead to pollen transfer from the scopa to the rest of the body and vice versa. Bees were then taken to the laboratory for identification, if needed with the help of European specialists. Identified bees were placed in the pollinator collection of the IMEDEA (Mediterranean Institute for Advanced Studies; UIB-CSIC), in the Balearic Islands, Spain.

Identification of pollen carried by bee individuals

For palynological analysis, we sampled pollen grains from all the wild bee individuals collected at each study community. We sampled the pollen by gently touching the individual body with a fuchsin gel cube, which we then melted and mounted on a slide with fuchsin used as stain (Sawyer & Pickard, 1981; Dafni *et al.*, 2005). To allow the comparison of pollen carried by specialized pollen-carrying structures and the rest of the body, for each female bee, we collected and mounted separately pollen from the scopa (i.e., hind leg hairs for Andrenidae, Halictidae, Colletidae, and Apidae and the ventral collecting brush for Megachilidae) and from the rest of the body (head, thorax, wings, and dorsal surface of the abdomen). We did not collect pollen located in the mesothoracic legs and on the sides of the abdomen to avoid contamination by pollen from the scopa. For male individuals, which do not have scopa, pollen from all parts of the body was collected. Same was done with the nine female individuals belonging to the genera *Sphecodes*, *Hylaeus*, and *Nomada* which do not have scopa (Michener, 2000). To avoid contamination between individuals and between the scopa and the rest of the body for female individuals, the pollen collecting equipment was systematically and carefully cleaned between each pollen sampling. Given these precautions, we consider potential contaminations (e.g., on the flower or inside the net), if any, to be minimal.

We then identified the pollen contained on the slides at 100 and 400× magnification using a light microscope (Aixovert, 200). We carried out pollen identification by comparing their morphology with online pollen picture databases (PalDat–a palynological database (2000 onward, www.paldat.org) and PollenWiki, Stebler Th. (https://pollen.tstebler.ch/MediaWiki/index.php?title=Pollenatlas, accessed April 2021), as well as with our own reference collection containing the pollen of 139 plant species. We collected these pollen grains directly from open flowers in the field and were then mounted on slides with fuchsin gelatin. When it was not possible to identify pollen grains to the species level with a high degree of reliability (Faegri *et al.*, 1989), we used the phytosociological survey of the study communities to guide the identification. Thus, we assumed that the pollen collected on a bee specimen corresponded to plant species present in the study community at the moment of capture. Similarly, the plant species on which we captured the insect was on some occasions used to select a species among those of the same morphotype (Zhao *et al.*, 2019). If this was not sufficient for the accurate identification of pollen grains to the species level, we limited the identification to a morphological type of pollen (see Table S1 for pollen morphotypes), as commonly done in palynological studies (e.g., Jędrzejewska-Szmek & Zych, 2013; Vianna *et al.*, 2014; Zhao *et al.*, 2019). For mixed networks (i.e., merging visitation and pollen interaction data), 32% of the interactions were limited to pollen morphotypes, most of them corresponding to the Asteraceae family (Table S1).

To define the strength of an interaction, we used the number of pollen grains per slide as a proxy of the pollen carried by an individual bee. When a plant species was represented by less than five pollen grains, we considered the interaction between the bee and the plant species as "weak," whereas a "strong" interaction was considered otherwise. We used a slightly lower number of pollen grains than other authors to define weak interactions (Bosch *et al.*, 2009; Zhao *et al.*, 2019), because some plant species in our communities seemed to be transported always in very small quantities (e.g., 5–10 grains per bee individual was typical for some Caryophyllaceae, authors' personal observation). Broken or immature pollen grains were not recorded during pollen counts, and not either pollen from non-entomophilous plants, such as *Pinus* sp., *Olea europaea*, *Juniperus* sp., or *Quercus ilex*, which can be found occasionally on the bees. We defined "pollen richness" as the total number of different plant species or pollen morphotypes detected on a bee individual.

Network analyses

We constructed quantitative visitation and pollen-transport networks for the eight study communities, each interaction being weighted by the number of times individuals of a particular pollinator species were found to interact with a plant species. We did not weight pollen interactions using the number of pollen grains (Alarcón, 2010), because it is an extremely time-consuming task and because plant–pollinator interactions can result in a highly variable pollen exchange (Sazatornil *et al.*, 2016; Ramírez-Burbano *et al.*, 2017; De Manincor *et al.*, 2020). We included in the networks all apoid specimens collected (representing 48 species) except *Apis mellifera*, which was not included because we aimed to focus on wild bees and, therefore, we did not collect this species systematically while sampling. For each of the eight study communities, we constructed five different network types describing plant–pollinator interactions using five different methods as follows: (1) "Visitation networks" built based on bee–plant interactions observed directly in the field. To allow the comparison between these networks and pollen-transport networks, in visitation networks we also grouped the plant species with similar pollen morphotypes that could not be differentiated using pollen analysis (see Table S1 for pollen morphotypes); (2–4) "Pollen-transport networks" were constructed with the interactions observed by means of pollen analysis. We constructed three types of these networks: (2) "Scopa pollen networks," only including information from the pollen collected in the scopa, (3) "Body pollen networks," only including the pollen sampled from the rest of the body (wings, head, front legs, thorax and dorsal side of abdomen), and (4) "All pollen networks," including the pollen collected from both the scopa and the other body parts. Lastly, we built (5) "Mixed networks," gathering all the interactions determined for an individual (both by visitation and pollen analysis). When we detected the same interaction between an individual and a plant species by different methods (i.e., field data, pollen in the scopa, and/or pollen in the body), we unified these data by considering the interaction only once.

To assess the effect of the method used to sample interactions on the structure of interaction networks, we built an interaction matrix for each study community and each method, with the pollinator species in rows, the plant species in columns, and each matrix cell containing the weight of the respective interaction. For each network, we calculated several metrics using *networklevel* and *metaComputeModules* functions from the bipartite R package v.2.16 (Dormann *et al.*, 2009; Dormann, 2011). To describe network size, we used the (1) plant species and (2) interaction (link) richness of each network. These two metrics assess the diversity of plant species used by bees for their nutrition (diet breadth) as well as the diversity of pairwise bee–plant interactions and, therefore, allow assessing the extent to which the sampling method affect network size (Bosch *et al.*, 2009; Pornon *et al.*, 2017). Since palynological analyses may allow us to sample more interactions, a higher value for each of these two first metrics is expected in pollen-transport networks than in visitations networks. These metrics could further increase in mixed networks by including bee–plant interactions that do not result in pollen transfer (Bosch *et al.*, 2009). Second, we studied the effect of the interaction sampling methods on network structure. Network structure was described by using three commonly used network metrics: (3) network specialization (H_2'), (4) modularity, and (5) nestedness. Network specialization (H2') is a metric that ranges from zero (no specialization of the network) to one (full specialization of the network) (Blüthgen *et al.*, 2006; Dormann *et al.*, 2009). This metric was used to assess whether specialization in networks might be overestimated in visitation networks due to hidden interactions. Therefore, we expected network specialization to decrease in pollen-transport networks as palynological analysis may reveal rarer interactions increasing the generalist character of species. Modularity was calculated using the Beckett algorithm (Beckett, 2016), which measures the extent to which pollination networks are organized into subsets of strongly interlinked species weakly connected to the rest of the networks (Guimerà & Nunes Amaral, 2005; Olesen *et al.*, 2007; Watts *et al.*, 2016). The assumption of variation in modularity is more complex to predict. If additional interactions occur intramodules, then modularity will increase; otherwise inter-module interactions will decrease modularity. Nestedness measures the tendency of specialist species to interact preferentially with generalist ones (Bascompte *et al.*, 2003; Bascompte *et al.*, 2006). To calculate it, we used weighted NODF (Nestedness metric based on Overlap and Decreasing Fill), a quantitative index in which high values indicate higher nestedness (Almeida-Neto *et al.*, 2008; Almeida-Neto & Ulrich, 2011). As a decrease in the specialization is expected when using pollen loads, we expected to observe a decrease in nestedness.

Raw values of structural network metrics (network specialization, nestedness and modularity) may describe actual observed changes in network structures with the type of interaction sampling. However, to understand whether the structures found are different from those expected at random just based on the relative abundance of network components, the standardization of the metrics is needed. Thus, to standardize structural network metrics (network specialization, nestedness and modularity) by controlling for network size and connectance (Fründ *et al.*, 2016; Dalsgaard *et al.*, 2017), we also calculated z-scores ($z = [x - \mu]/\sigma$, where x is the observed value, and μ and σ the mean and standard deviation respectively of 1000 random networks—100 in case of modularity), following the *vaznull* null model (Vázquez *et al.*, 2007). This null model randomizes individual interactions in the network while maintaining the original values of connectance and network size. Null models were created using the function *nullmodel* from the bipartite R package v.2.16 (Dormann *et al.*, 2009; Dormann, 2011). z-scores compare the observed network metric to the distribution of simulated metrics; thus, a negative value indicates a metric that falls below what is expected at random, and a positive value reflects one that falls above. As for raw metrics, the z-scores obtained were then compared between network types to confirm that the observed changes were not solely driven by variations in network size and connectance.

To evaluate the role of sex structuring the networks, we built two interaction matrices based on the total number of interactions recorded (using both visitation and pollen data) for each study community, with bee individuals in rows and plant species in columns. The first set of matrices included information of all the pollen collected on the individuals (both in the body and in the scopa, if they had it), while the second set included only the pollen collected on the body of bees. For all individuals, we calculated: (1) individual specialization, as d' (Blüthgen *et al.*, 2006; Dormann, 2011), which varies from 0 when an individual is strongly generalist (it visits a large number of species also visited by other species) to 1 that indicates maximum specialization (the pollinator visits a narrow subset of species not visited by other pollinators); and (2) closeness centrality (Freeman, 2002), which estimates the proximity of an individual to all others in the networks through shared resources (Martín-González *et al.*, 2010). These metrics were calculated respectively using the *specieslevel* and *CC* functions of the bipartite R package v.2.16 (Dormann *et al.*, 2009; Dormann, 2011).

Statistical analysis

All the statistical analyses presented here were conducted in R software v.4.0.5 (R Core Team, 2020) and correspond to generalized mixed models (GLMMs; *glmer* function in lme4 R package v.1.1.27; Bates *et al.*, 2015) or linear mixed models (LMMs; *lmer* function from the nlme R package v.3.1.152; Pinheiro *et al.*, 2020), depending on the nature of the response. To analyze differences in pollen loads between body parts (scopa vs. rest of body) in females, we performed three separate GLMMs with the following response variables: (1) the efficiency of pollen transfer to pollinators, by checking whether the interactions observed in the field were confirmed by the pollen grains found on individuals, using a Binomial model (confirmed vs. non-confirmed); (2) the richness of interactions per individual (hereafter pollen richness), using a Poisson distribution after checking for the absence of overdispersion (Zuur *et al.*, 2009); and (3) the strength of interactions using the proportion of "weak" (less than five pollen grains of the species) and "strong" (five or more pollen grains of the species) interactions, again with a Binomial distribution (weak vs. strong). In these models, the body part was the categorical predictor variable, and the study community and the individual identity nested within species were included as random factors to control for pseudoreplication (Zuur *et al.*, 2009).

To compare pollen loads between sexes we conducted also separate GLMMs for the same response variables as to study body parts (efficiency in pollen transfer, pollen richness, interaction strength). In these models, though, the sex was included as a predictor variable and both the study community and the species were included as crossed random factors; we did not include the individual identity within the random effect as in this case there was no pseudoreplication within female individuals. Two set of models were conducted to compare pollen loads in males and females: (i) using all the pollen grains recorded (both in scopa and rest of the body for females) and (ii) considering only the pollen collected on the body (i.e., excluding scopa pollen, as males do not have it). In this way, we could compare differences between sexes in plant-pollination and bee-feeding interactions. The analyses presented in the main text were conducted with all the specimens collected; however, analyzing only the 13 species for which we have both sexes did not change the results (results shown in Supporting Information).

To analyze the effect of the methodology used to sample interactions on network structure, we run different GLMMs or LMMs where the response variables (in separate models) were the different network-level metrics (both raw metrics and *z*-scores) and the predictive variable was the type of network built (i.e., Visitation, Scopa pollen, Body pollen, All pollen, and Mixed networks). All these models included the study community as random factor to control for pseudoreplication (Zuur *et al.*, 2009). Due to the nature of the data, we used (1) Poisson distribution for the analyses of plant species and interaction richness, after checking for the absence of overdispersion (Zuur *et al.*, 2009); (2) Gaussian distributions for the analyses of raw nestedness, and of *z*-scores of network specialization and modularity, after evaluating normality using Lilliefors test with the nortest R package v.1.0.4 (Gross & Ligges, 2015); and (3) Gamma distributions with log link function for the models of raw modularity and network specialization (H_2'), and of *z*-score of nestedness.

Finally, to assess the role of bee sex within the networks, we run GLMMs in which individual-level specialization (d') and position in the networks (closeness centrality) were the response variables and bee sex the predictive variable. Due to the nature of the data, we used a Gamma distribution (link = log) for both models and included the study community and species as crossed random factors to control for pseudoreplication (Zuur *et al.*, 2009).

For all models, we calculated the significance of predictive variables based on Likelihood Ratio Tests using the *Anova* function from the car R package v.3.0.10 (Fox & Weisberg, 2019), followed by *post hoc* analyses using the *emmeans* function from the emmeans R package v.1.6.0 (Lenth, 2020). Bar graphs and boxplots were performed using the ggplot2 R package v.3.3.3 (Wickham *et al.*, 2016), and networks were plotted using the *plotweb* function from the bipartite R package v.2.16 (Dormann *et al.*, 2009; Dormann, 2011).

Results

In the eight study communities, we collected a total of 255 bee individuals by hand-netting, 248 in spring (45 males and 203 females) and 7 in autumn (3 males and 4 females). Number of collected individuals per study community ranged from 12 to 54 (for individuals collected per community, see Table S2). These individuals belonged to 48 different species (Apidae 6; Megachilidae 13; Andrenidae 10; Halictidae 12; and Colletidae 7; see species and abundance per species and sex in Table S3). All captured individuals had pollen on their bodies except for two males and one female. Using pollen analysis on the pollen loads carried by the bees, we determined 1203 interactions (based on visitation or pollen data and

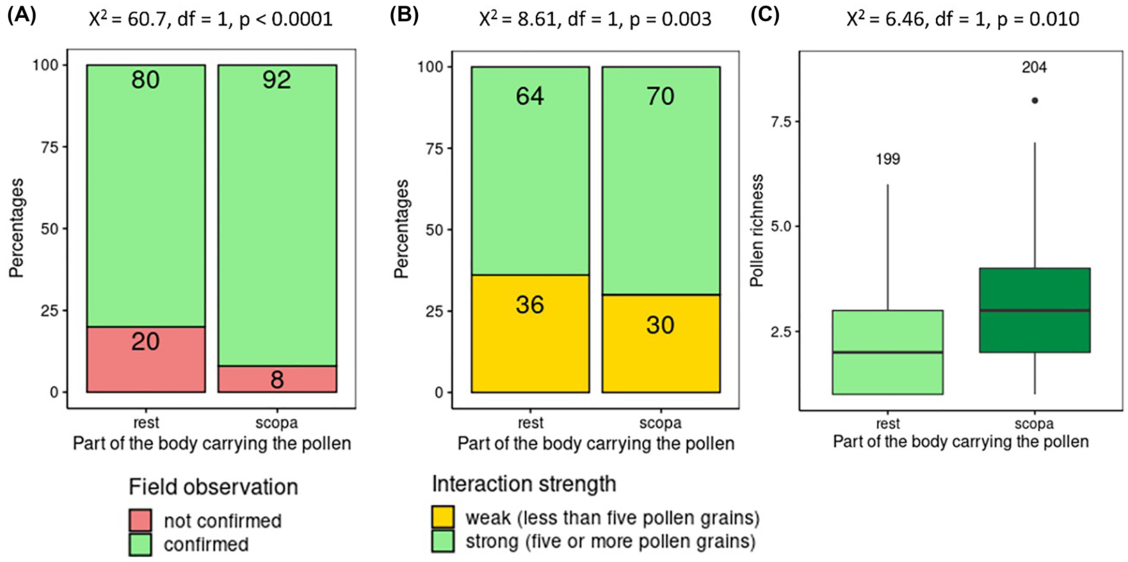

Fig. 1 Pollen loads in scopa vs. rest of the body in female bees. (A) Percentage of interactions observed in the field that were confirmed by pollen analysis. (B) Percentage of weak interactions (less than five pollen grains of a plant species carried by an individual bee) and strong interactions (five or more pollen grains carried). (C) Pollen richness (i.e., number of different plant species or pollen morphotypes) carried by individuals. Female pollen was split into pollen from the scopa (scopa) and from the rest of the body (rest). Values above the boxplots reflect the number of individuals included in each group.

without duplication per individual, i.e., each individual could only be linked once to a plant species) between bee individuals and plants (Table S2 for plant and interaction richness detected with each method), 156 for males, 570 for females when analyzing pollen from the scopa, and 477 for females when analyzing the rest of the body. By combining this information with the 255 interactions observed in the field, we were able to describe interactions between wild bees and 76 species and 14 pollen morphotypes (Table S1) of entomophilous plants. The highest pollen richness per individual bee was found in two females and one male and was of nine plant species; maximum pollen richness in the scopa was eight, and six in the rest of the body. Pollen richness was positively related to the abundance of bee species in the visitation networks (Fig. S2 and Table S4). Table S5 indicates the sample sizes for all the statistical analyses reported below.

Pollen loads in different body parts of female bees: scopa vs. rest of the body

The pollen loads in the scopa allowed to confirm a greater number of interactions observed in the field than the pollen collected on the rest of the body of female bees (92% of interactions confirmed vs. 80%, respectively; $\chi^2 = 60.7$, df = 1, $P < 0.0001$; Fig. 1A). In addition, the analysis of pollen in the scopa indicated overall stronger interactions with plants, as shown by a lower proportion of weak interactions (i.e., those interactions that resulted in less than five carried pollen grains), compared to the analysis of pollen in the rest of the body (30% vs. 36%, respectively; $\chi^2 = 8.61$, df = 1, $P = 0.003$; Fig. 1B). Finally, the pollen loads in the scopa were also richer in species or pollen morphotypes than the pollen loads found on the rest of the body (mean ± SE: 2.79 ± 0.10 vs. 2.40 ± 0.09 species or morphotypes/individual, respectively; $\chi^2 = 6.46$, df = 1, $P = 0.01$; Fig. 1C).

Differences in pollen loads between apoid sexes

The distribution of the sexes in our bee sample was unbalanced, with 19% of the individuals being males and 81% females. The results shown here correspond to the analyses of all specimens collected, however, the same results were obtained when only the 13 species for which we had data on females and males were analyzed (Table S6). When considering the total amount of pollen carried by individual bees, there was a much higher proportion of interactions observed in the field (visitation data) that did not result in any pollen transport (pollen data) in males than in females (27% vs. 8%, respectively; $\chi^2 = 10.6$, df = 1, $P = 0.001$; Fig. 2A). This significant difference between sexes was maintained (27% vs. 20%, respectively; $\chi^2 = 134\,932$, df = 1, $P < 0.0001$; Fig. 2B) when

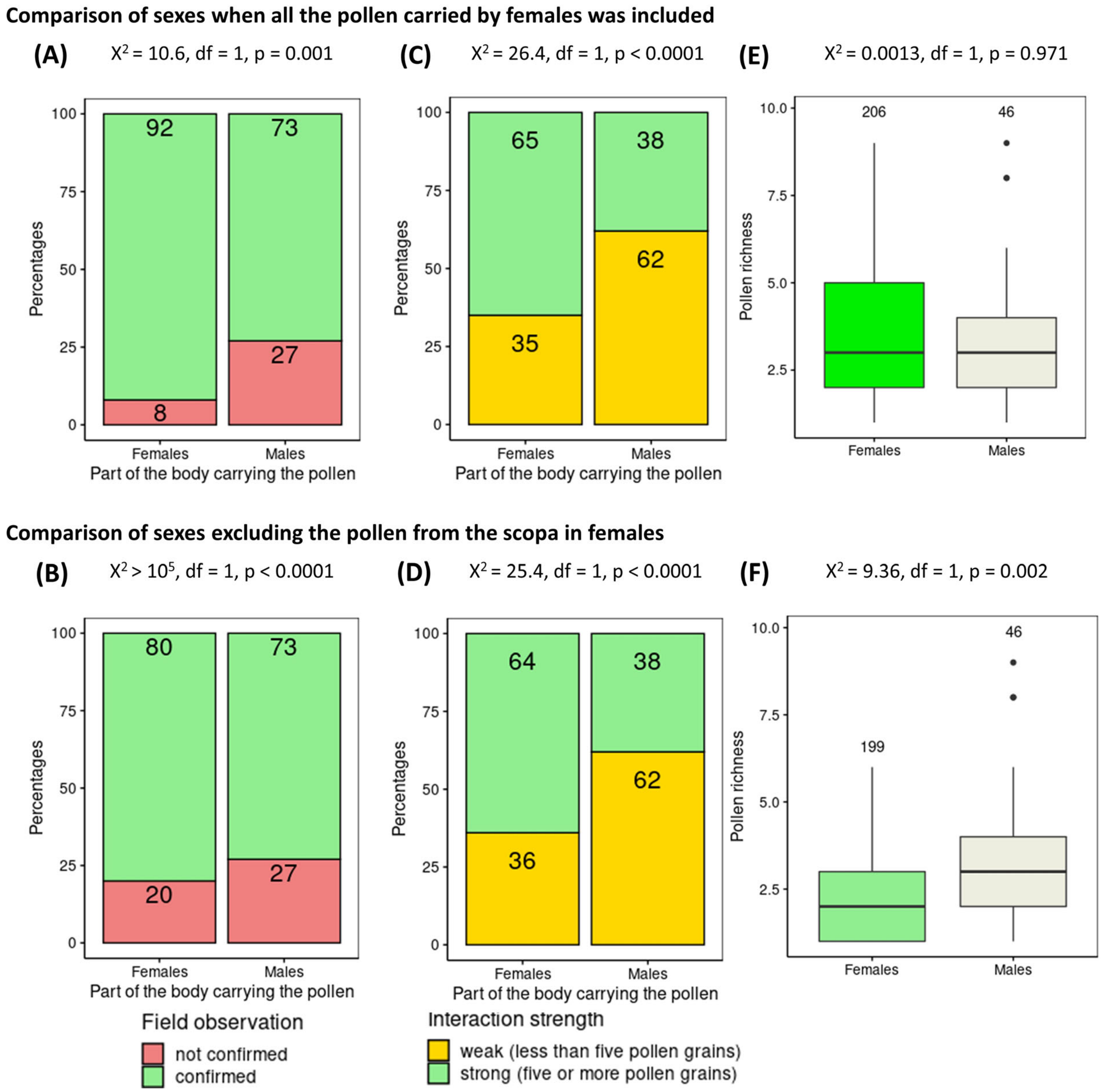

Fig. 2 Differences between bee sexes in carried pollen loads. Percentage of interactions observed in the field that were confirmed by pollen analysis, when including the pollen loads of scopas (A) and when excluding them (B) for female bees. Percentage of weak interactions (less than five pollen grains of a plant species on a bee individual) and strong interactions (five or more pollen grains carried), when including the pollen loads of scopas (C) and when excluding them (D) for female bees. Pollen richness (i.e., number of different plant species and pollen morphotypes) carried by individuals, when including the pollen loads of scopas (E) and when excluding them (F) for female bees. Values above the boxplots reflect the number of individuals included in each group.

we excluded the pollen from the scopa and compared only the pollen carried on the rest of the body (wings, head, front legs, thorax, and dorsal side of abdomen).

We found a higher proportion of weak interactions (less than five pollen grains) in males than in females (62% vs. 35%, respectively; $\chi^2 = 26.4$, df = 1, $P < 0.0001$; Fig. 2C) when all the pollen found on the bees was considered. Interestingly, when scopa pollen was excluded, the differences between males and females in the proportion of weak interactions were still significant (62% vs. 36%, respectively $\chi^2 = 25.4$, df = 1, $P < 0.0001$; Fig. 2D).

The richness of pollen carried by individuals was not significantly influenced by the sex of bees, males and females carrying similar pollen richness (mean ± SE: 3.39 ± 0.30 vs. 3.39 ± 0.12 species or morphotypes/individual, respectively; $\chi^2 = 0.001$, df = 1, $P = 0.971$; Fig. 2E) when all the pollen found on the bees was analyzed. However, when we excluded the pollen loads carried in the scopa, the pollen transported on the rest of the body was significantly richer in males than in females (mean ± SE: 3.39 ± 0.30 vs. 2.40 ± 0.09, respectively; $\chi^2 = 9.36$, df = 1, $P = 0.002$; Fig. 2F). Seven females had pollen only in their scopa and not on the rest of their body. The number of female individuals included in this second analysis was therefore slightly lowered.

Network comparison: visitation, pollen-transport networks, and mixed networks

Fig. 3 illustrates with an example the observed changes in network complexity depending on the sampling method used. The two parameters describing the size of our networks were significantly affected by the interaction sampling method. Both plant species richness ($\chi^2 = 81.5$, df = 4, $P < 0.0001$; Fig. 4A) and interaction richness ($\chi^2 = 230$, df = 4, $P < 0.0001$; Fig. 4B) were lower in visitation networks than in all other network types. Regarding plant species richness, the different pollen-transport networks did not differ significantly from each other or from the mixed networks. However, mixed networks contained more interactions than either the scopa or body pollen-transport networks individually.

For all the metrics evaluated, we found significant differences between visitation networks (based on field observations) and all other networks constructed including the information of pollen loads. Visitation networks showed higher specialization (H2'; $\chi^2 = 255$, df = 4, $P < 0.0001$; Fig. 5A) and modularity ($\chi^2 = 111$, df = 4, $P < 0.0001$; Fig. 5B) but lower nestedness ($\chi^2 = 41.7$, df = 4, $P < 0.0001$; Fig. 5C) than all the pollen-transport and the mixed networks. Mixed and all pollen networks (merging pollen from the scopa and the rest of the body) did not show significant differences in any of the metrics (Fig. 5). The scopa pollen and body pollen networks did not show either significant differences with all pollen networks and mixed networks regarding the metrics. The comparison of *z*-scores revealed similar results to the raw metrics with a higher specialization ($\chi^2 = 27.9$, df = 4, $P < 0.0001$; Fig. 5D) and modularity ($\chi^2 = 75.7$, df = 4, $P < 0.0001$; Fig. 5E) of visitation networks than all pollen-transport and mixed networks. However, nestedness no longer varied significantly with network type ($\chi^2 = 5.14$, df = 4, $P = 0.273$; Fig. 5F).

Sex effects on the specialization and position of individuals in networks

When considering all the pollen carried by individuals, males showed higher specialization in the networks (d') than females (mean ± SE: 0.35 ± 0.03 vs. 0.24 ± 0.01, respectively; $\chi^2 = 14.7$, df = 1, $P = 0.0001$; Fig. 6A). Accordingly, closeness centrality was found to be higher in females, but the difference was marginally non-significant (mean ± SE: 0.028 ± 0.002 for males vs. 0.032 ± 0.001 for females; $\chi^2 = 2.93$, df = 1, $P = 0.090$). When only considering the pollen carried on the body for males and females, males still showed a higher specialization (d') than females (mean ± SE: 0.36 ± 0.03 vs. 0.27 ± 0.02, respectively; $\chi^2 = 10.6$, df = 1, $P = 0.001$; Fig. 6B), but we did not find any difference between sexes regarding closeness centrality (mean ± SE: 0.030 ± 0.002 for males vs. 0.032 ± 0.001 for females; $\chi^2 = 0.24$, df = 1, $P = 0.622$).

Discussion

Palynological analyses uncovered a much higher number of pollination interactions than these found with field observations only, which affected the composition and organization of networks. The pollen located in specialized structures of female bees was richer and represented more interactions observed in the field than the pollen located on the rest of the body. Despite these differences in pollen loads, including the interactions detected on one or another part of the body had little influence on network structure. Female bees carried pollen in much larger quantities than males, confirming more of the observed field interactions and showing higher interaction strength; consequently, females had a stronger role structuring the interactions at the community level.

Pollen loads in different body parts of female bees

Most female bees have collecting brushes or scopa that allow them to gather pollen in large quantities to feed the larvae (Michener, 2000). The pollen placed on the body of the bee is periodically deposited in this scopa by the bee, constituting a pollen pool on the individuals (Thorp, 2000; Danforth *et al.*, 2019). While the vast majority of the pollen on females is present in the scopa, pollen outside in the rest of the body appears in smaller

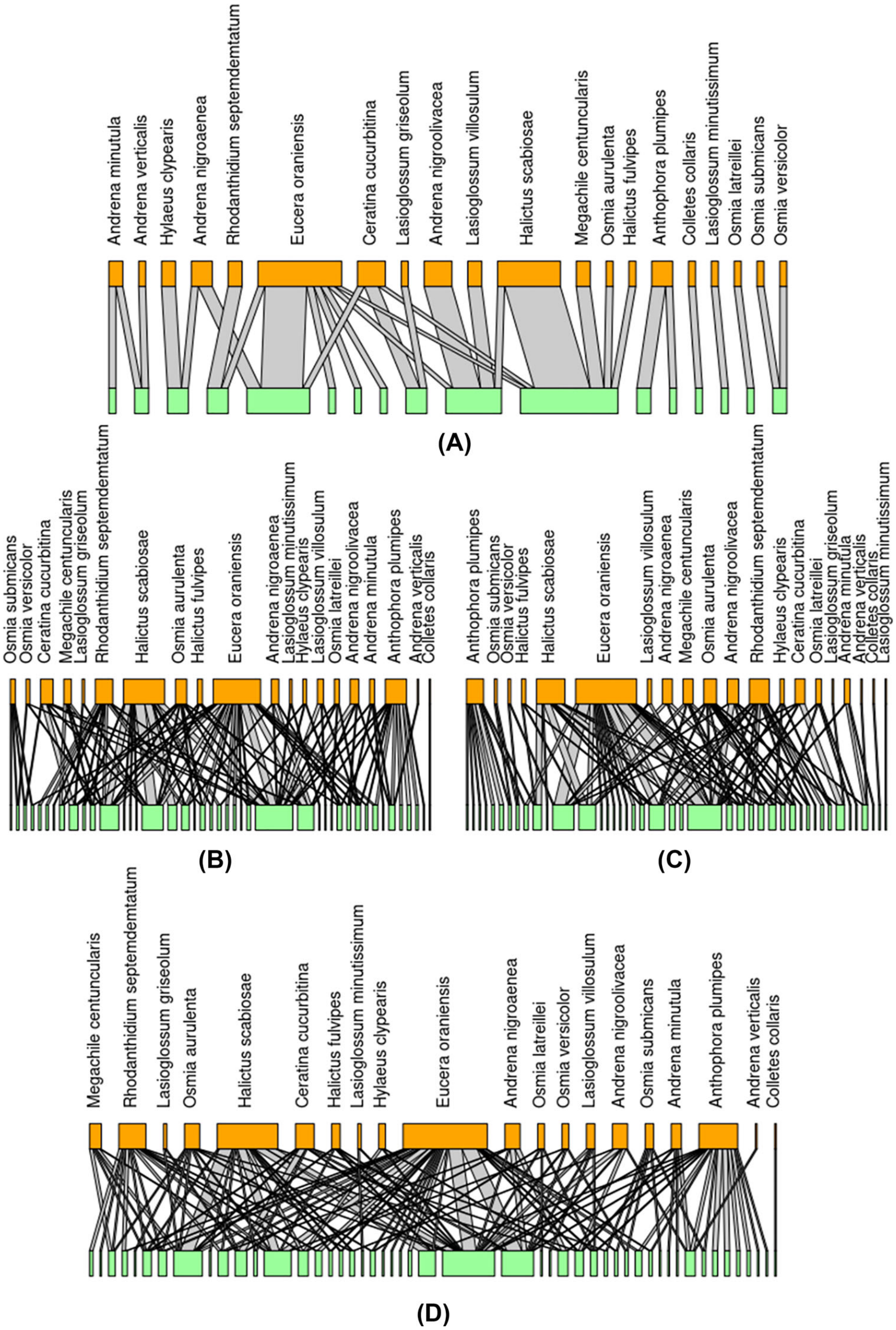

Fig. 3 Illustration of networks built with different interaction sampling methods for one of the study communities (s'Heretat). (A) Visitation network, (B) scopa pollen network, (C) body pollen network, and (D) mixed network. All bee species are depicted in orange, plant species are in green, gray lines represent interactions between the two groups, and their thickness is proportional to the frequency of the interaction. Plant species names are not displayed for the simplicity of the figure.

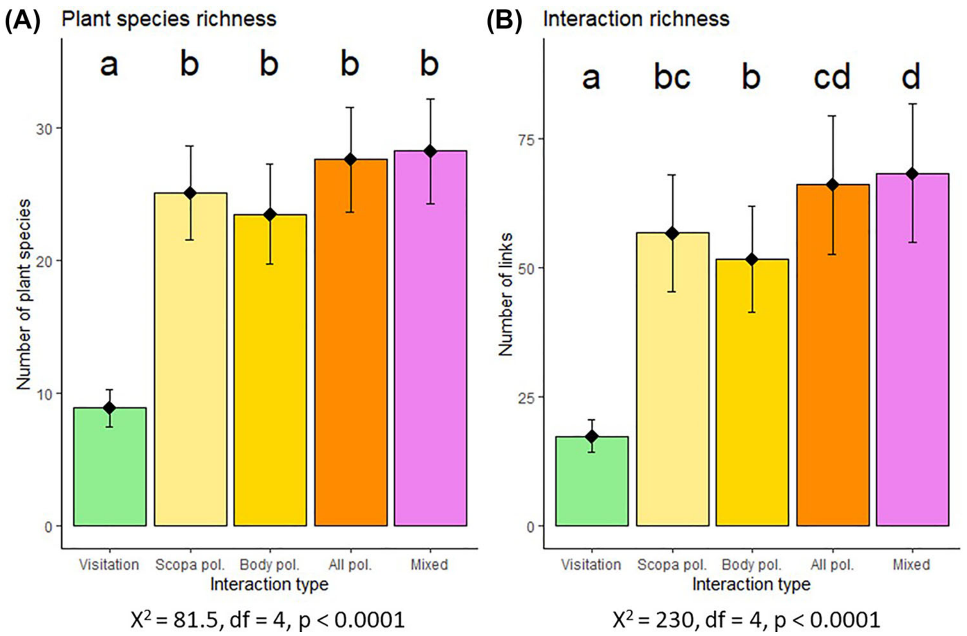

Fig. 4 Mean (± SE) plant richness (A) and interaction richness (B) for each method of interaction sampling. "Visitation" refers to networks built using field observations; "pol." refers to networks based on palynological analyses containing pollen from the scopa only ("Scopa pol."), from the rest of the body only ("Body pol.") or from all the specimen (i.e., both in the scopa and the rest of the body, "All pol."). "Mixed" refers to networks containing all the interactions of the previous methods. Different letters above the bars indicate significant differences between methods after post hoc tests.

quantities. However, the pollen in the scopa is compacted and poorly available for pollination (Michener, 2000; Alarcón, 2010). Dense scopas (e.g., in *Apis mellifera* and other corbiculate bees) can minimize pollen availability for pollination and even reduce the fruit and seed set of visited plants (Parker *et al.*, 2015). Therefore, it becomes necessary to study the differences between pollen loads in these structures and in the rest of the body, to understand which proportion of interactions is kept by the bee to feed its offspring and which proportion result in pollen transport available for pollination and ultimately result in fruit and seed production. This may also show whether each side of this plant–bee mutualism benefits from the interaction symmetrically and helps to understand the extent to which plant-pollination (body pollen) and bee-feeding (scopa pollen) interaction networks may differ. We found that only 92% and 80% of interactions observed in the field could be confirmed with pollen analysis of scopa and body pollen, respectively. This result agrees with previous studies indicating that even in apoids, a part of the interactions with pollinators does not result in any pollen transport (Zhao *et al.*, 2019). In addition, pollen analysis indicated that a considerable part of the observed field interactions was weakly supported (less than five pollen grains carried), particularly when we only analyzed the pollen carried on the body. This result, together with the higher pollen richness we found in the scopa compared to the rest of the body, suggests that the behavior of pollen deposition in the scopa might significantly reduce the number of available pollen grains to a level that may no longer be sufficient to ensure pollination in some plant species (Parker *et al.*, 2015). Overall, our results warn against the exclusive use of floral visits to reflect pollen transfer activity by the pollinators as some field interactions do not result in pollen transfer (Zhao *et al.*, 2019). Floral visits reflect more the nutrition of the imago pollinator (nectar) and its larvae (pollen) than the pollination activity it supports. Plant-pollination networks, based on body pollen and reflecting the pollen available for pollination, should thus contain a lower diversity of interactions than bee-feeding networks based on scopa pollen, which cannot result in pollen transfer to plants.

The importance of bee sex in pollen transport

Many fewer males were observed visiting flowers than females. This difference was the expected result of the collection method, as we collected individuals coming into contact with the floral reproductive structures, which

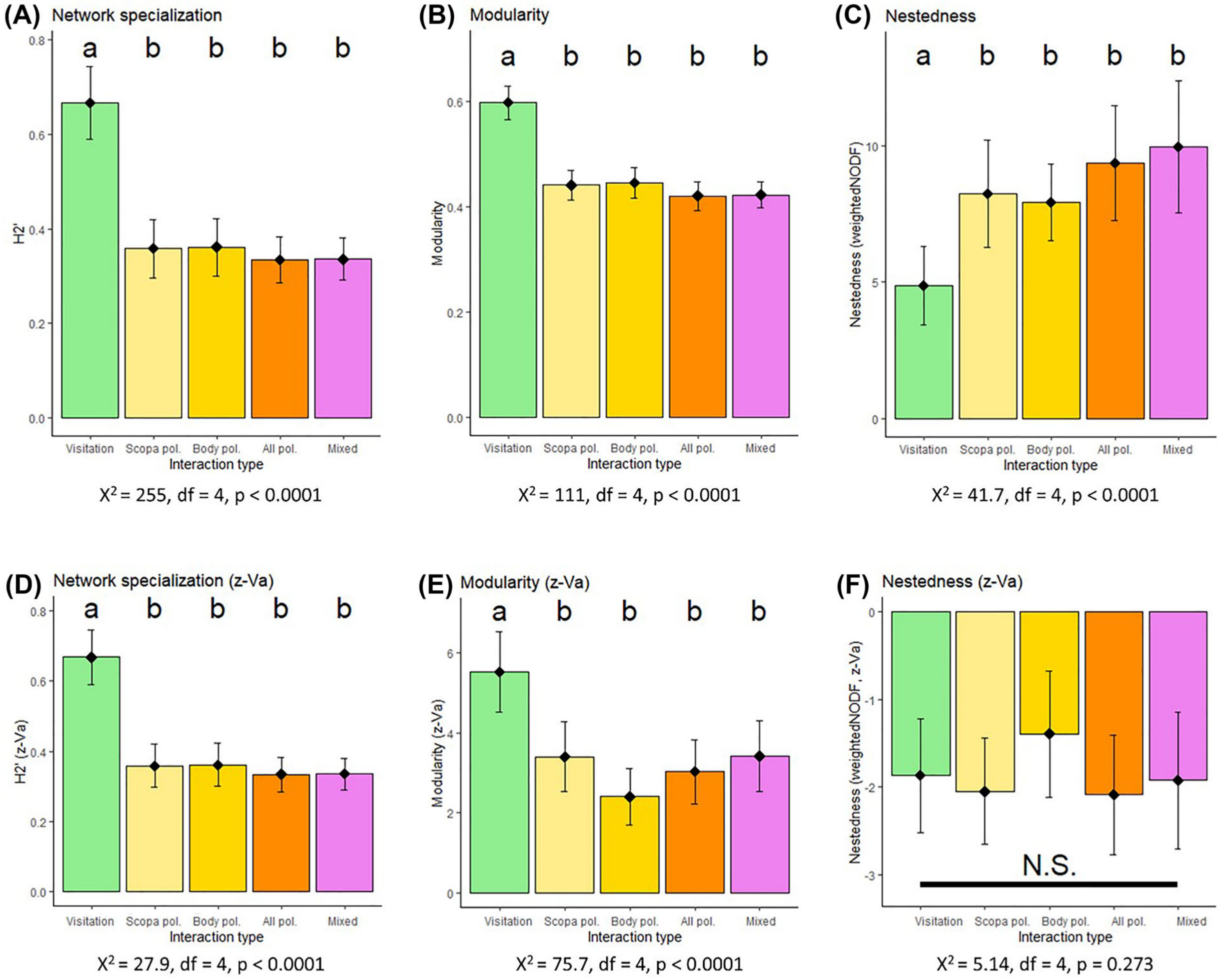

Fig. 5 Mean (± SE) of network-level metrics (A–C) and their *z*-scores (D–F) for each method of interaction sampling. (A, D) network specialization, (B, E) modularity, and (C, F) nestedness. "Visitation" refers to network built using field observations; "pol." refers to networks based on palynological analyses containing pollen from the scopa only ("Scopa pol."), from the rest of the body only ("Body pol.") or from all the specimen (i.e., both in the scopa and the rest of the body, "All pol."). "Mixed" refers to networks containing all the interactions of the previous methods. "*z*-Va" refers to the *z*-scores calculated using *vaznull* null models (Vázquez *et al.*, 2007). Different letters above the bars indicate significant differences between methods after *post hoc* tests when the predictor variable had a significant effect; N.S. indicates non-significant effect of method (network type) on the studied metric.

females are more likely to do for longer time periods than males. We found significant differences between sexes regarding the number of interactions observed in the field that resulted in pollen transport. Females carried pollen from 19% more of the plants they were observed visiting than males. Females also supported 27% less weak interactions than males, and the difference was maintained when the pollen from the scopa in females was excluded (i.e., only considering the pollen available for pollination). Both results demonstrate that pollen-transport efficiency is higher in females than in males and that female bees have a higher role in the pollination of flowering plants. However, although females and males carried similar pollen richness overall (including scopa and rest of the body pollen for females), males carried a higher richness of pollen grains when only the pollination-available pollen was analyzed (excluding scopa pollen). These results are in line with the few studies that have compared bee sexes in pollination (Roswell *et al.*, 2019; Cullen *et al.*, 2021). In addition to having different floral preferences (Roswell *et al.*, 2019), our results show that females are better pollen carriers than males. Females acquire pollen more efficiently after a contact with the floral structure and support interactions with a larger

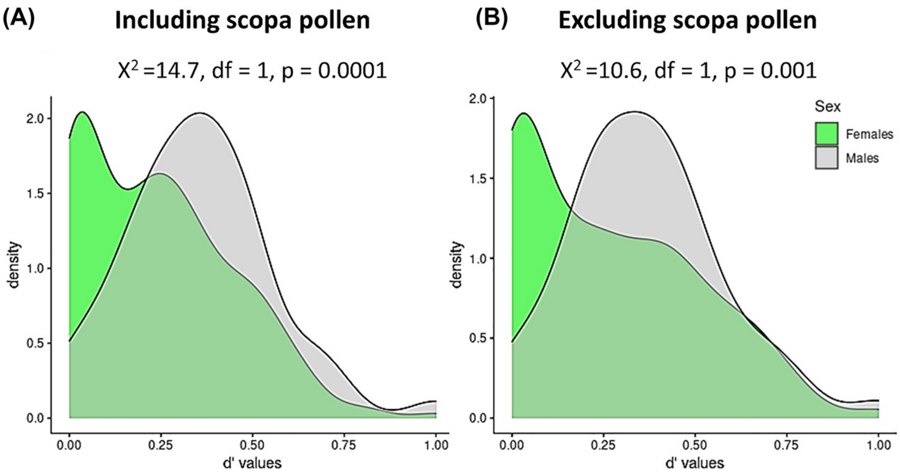

Fig. 6 Density plots depicting specialization level in networks (d') for bee sexes. The metric was calculated using all interactions detected, that is, merging data from field observations and pollen analysis (mixed networks). Specialization of individuals: (A) when all the pollen present on the individuals was considered and (B) when only the pollen present on the body was included (scopa excluded for female bees).

amount of pollen grains (Cullen *et al.*, 2021). These differences between males and females can be explained by their foraging pattern and behavior. Females actively collect pollen to feed larvae; they have therefore a much larger amount of pollen on their body (Willmer & Stone, 2004; Danforth *et al.*, 2019). This increases the amount of pollen grains retrieved after visiting a flower and allows for interactions supported by more pollen even excluding the scopa pollen. Regarding males, they do not collect pollen on purpose as they only feed on nectar. They therefore obtain pollen less easily after a flower visit and in smaller abundance. Finally, female bees do not interact with more plant species than males and the richness of their pollination-available pollen is even lower than in males. This higher pollen richness underlines male higher diversity of floral visits. Several factors may increase their diversity of interaction with plant species: (i) As males collect nectar only, they may be less exigent than females (who have to feed their larvae) regarding the nutritional quality of their food, and may feed on a larger diversity of plant species. (ii) In order to breed, males perform patrolling behavior in search of females and frequently land on surrounding flowers waiting for females, probably gathering accidentally the pollen of these species (Alves-dos-Santos, 1999; Alcock *et al.*, 2010; Danforth *et al.*, 2019). (iii) Furthermore, males of several solitary bees usually sleep on flowers while females do not (Pinheiro *et al.*, 2017; Danforth *et al.*, 2019). They might therefore carry the pollen of these dormitory flowers, which may not be visited by females. In the end, the higher pollen richness carried by the males may slightly increase heterospecific pollen deposition, leading to a decrease in plant fitness (Morales & Traveset, 2008). On the other hand, females carrying a lower pollen diversity but more pollen of each species make them more efficient pollinators.

Effects of sampling methodology on the structure of pollination networks

We found strong differences in structure between visitation and all other types of networks constructed including information on pollen loads, which agrees with the results of previous authors (Bosch *et al.*, 2009; Vianna *et al.*, 2014; Ramirez-Burbano *et al.*, 2017; Zhao *et al.*, 2019; Souza *et al.*, 2021). The inclusion of interactions detected by pollen analysis increased plant species richness in the network but also interaction richness (Bosch *et al.*, 2009). This is because, in contrast to single visitation observations, pollen analysis allows to uncover interactions performed over a longer period of time (e.g., pollen foraging time of 60 min for *Colletes hederae*, Michener, 2000; Bischoff *et al.*, 2005) before it is discharged to feed the larvae or pollinate the plant. In line with other authors (Bosch *et al.*, 2009; Vianna *et al.*, 2014), uncovering new and rarer interactions using pollen data also resulted in higher nestedness and lower network specialization (H2') than in visitation networks. This decrease in specialization warns against the overestimation of specialization levels when visitation networks are analyzed. Visitation networks were also more modular, indicating that the additional interactions uncovered by pollen analysis were mainly inter- rather than intramodular interactions. These variations in specialization and modularity remained valid in size standardized networks (*z*-scores), showing that these results cannot be attributed solely to the variation in network size. The differences between visitation and pollen-transport networks in nestedness, conversely, were no longer detected when *z*-scores were compared, indicating that the differences found in nestedness between these types of networks were due to differences in network size. Even though some interactions in the field did not result in pollen transport, adding the interactions observed in the field (visitation data) to the interactions detected during pollen analysis (all pollen data) to build mixed networks did not influence any studied network metric respect to those built exclusively with pollen data.

Our results evidence that the method used to sample interactions influences the structure of networks from which we derive ecological conclusions. The most representative and suitable networks for the analysis of plant–pollinator interactions may be pollen and mixed networks. Interactions observed in the field that do not result in any or only in weak pollen transfer can be retained in the network or not, depending on the aim of the research. If the study is focused on bee-feeding interactions, these interactions should be kept since they result in a nutritive nectar supply for the pollinator, covering the other (often neglected) side of the mutualism. However, if only plant pollination is evaluated, then these interactions are no longer of interest as they must be viewed as pollinator cheating on the plant (Zhao *et al.*, 2019).

Spotlight on pollen-transport networks, whether or not to include the pollen from the scopa

Despite the differences in pollen loads between the scopa and the rest of female bodies, there was no significant variation between the structure of the different pollen-transport networks when including or excluding the pollen in the scopa. Pollen-transport networks based on palynological data frequently do not include the scopa pollen loads of female bees (e.g., Alarcón, 2010; Souza *et al.*, 2021) because this pollen is enclosed and less available for plant pollination than the pollen found in the rest of the body (Michener, 2000; Alarcón, 2010). Nevertheless, scopa pollen is still crucial from the pollinator's point of view, as it constitutes the main nutrients provided to the larvae (Michener, 2000). The use of either source of pollen grains in networks allows to highlight different facets of the plant–pollinator relationship. Excluding pollen from the scopa allows the only inclusion of pollen available for pollination and reflects plant pollination networks. Considering only the pollen of the scopa reflects the pattern of interaction allowing the nutrition of the bee larvae whereas considering the whole pollen allows to additionally integrate interactions carried out by adult bees to feed themselves (nectar) and to represent general bee-feeding networks. The lack of significant variation in metrics between these different types of pollen-transport networks may suggest that the structure of plant-pollination and bee-feeding interaction networks are similar and confirm that bees do pollinate the plants they feed on and on which they collect pollen to feed the larvae. However, our networks were weighted using the number of individuals of a bee species interacting with a plant species (same as done by other authors, e.g., Souza *et al.*, 2021), not by using the number of pollen grains of each plant species on each individual (as in Alarcón, 2010). As the pollen in the scopa is much numerous than pollen carried on the rest of the body, we cannot discard larger differences between network structures when including and excluding the scopa pollen if the number of pollen grains would have been considered. Knowing this, we suggest using (1) only the pollen present on the body of bees (outside the scopa) to represent the pollination activity and (2) the whole pollen set or even considering mixed networks to represent the bee-feeding interactions.

Sex effects on the role of individuals in networks

The major difference in pollen loads and associated interactions observed between sexes leads to think in a different role of bee sexes in the pollination networks. Although males and females carried the same pollen richness overall, our results indicate that male bees are more specialized (d' index) in the networks than female bees, and this regardless of whether the pollen in the scopa is considered or not in female bees. This is because d' measures the extent of specialization of a pollinator species based on its interaction frequencies and the interaction frequencies of the rest of pollinators in the network (Bluthgen *et al.*, 2006). Females are more generalists than males because they visit more frequently many species also visited by other individuals in order to feed efficiently on abundant and rewarding species. Also in this line, closeness centrality, that is a measure related to generalization that indicates the proximity between one individual and the others in the community (Martín-González *et al.*, 2010), tended to be higher in females. However, these differences were marginally non-significant, maybe because we used networks not weighted by the amount of pollen grains carried by the individuals. In addition to their generalist character, as a high closeness centrality is associated with a greater role on network stability (Martín-González *et al.*, 2010), our results suggest that females overall tend to participate more in network-wide stability than males.

Pollination networks are often analyzed at the specific level; however, it has been shown that intraspecific differences may be responsible for different ecological outcomes (Bolnick *et al.*, 2011; Zwolak, 2018). Consequently, some authors have demonstrated the importance of considering other layers of detail, including the individual role in networks (Tur *et al.*, 2014; Valverde *et al.*, 2016). Agreeing with this, our results show the interest of going beyond the specific level to better understand the role of bee sexes in plant-pollination networks or bee-feeding networks. Future work may consider intraspecific differences in morphological traits to advance further in our understanding of the role of sex in mutualistic networks.

Conclusion

Palynological data uncovered a large number of additional interactions not detected in the field, leading to profound changes in the structure of networks. However, although pollen loads in the scopa of females were richer and more abundant than those found on the rest of the body, the inclusion of pollen from the scopa when building the networks did not significantly influence their structure. The interactions between females and plants were stronger but not richer than those of males. In the networks, this resulted in more generalist female bees, which also tended to be more central in the networks compared to males, indicating a stronger role of females structuring the networks. Our work highlights the considerable advantage of using palynological data to increase the resolution of networks but also to understand relevant ecological questions such as the role of sexes in pollination or the difference between plant-pollination and bee-feeding interaction networks.

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Disclosure

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Supporting Information

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

Fig. S1 Map of the location of the eight study communities across Mallorca Island. The inset shows the location of the island within the west of the Mediterranean basin.

Fig. S2 Relationship between bee species abundance in visitation networks and the pollen richness it carried: considering both weak and strong interactions including (A) and excluding (B) *Eucera oraniensis* (the most abundant species), and only considering strong interactions including (C) and excluding (D) *Eucera oraniensis*.

Table S1 Lists of species included in the different pollen morphotypes when the identification to the species level was not possible.

Table S2 Number of specimens collected, and plant and interaction richness (i.e., number of plant species/morphotypes and pairwise bee-plant interactions, respectively) determined by each sampling method for each study community.

Table S3 List of bee species and number of specimens by sexes and in total for each species collected.

Table S4 Results of the GLMMs conducted to test the relationship between bee species abundance in visitation networks and the pollen richness it carried: considering both weak and strong interactions including (A) and excluding (B) *Eucera oraniensis* (the most abundant species), and only considering strong interactions including (C) and excluding (D) *Eucera oraniensis*. The models used a Poisson distribution and included the species as a random factor.

Table S5 Sample sizes for the different statistical analyses included in this study.

Table S6 Results of the GLMMs conducted to compare pollen loads between bee sexes including only the 13 species for which specimens of both sexes were collected (*Andrena fabrella*, *Andrena flavipes*, *Andrena nigroaenea*, *Andrena nigroolivacea*, *Andrena sardoa*, *Anthophora canescens*, *Eucera oraniensis*, *Halictus gemmeus*, *Lasioglossum griseolum*, *Lasioglossum villosulum*, *Megachile centuncularis*, *Megachile sicula and Rhodanthidium septemdentatum*).