RESEARCH PAPER

Landscape effects on pollination networks in Mediterranean gypsum islands

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ABSTRACT

- Habitat fragmentation is a major driver of global change that has operated historically on Mediterranean ecosystems. However, more needs to be understood about how fragmentation influences ecological interactions, particularly pollination. Gypsum outcrops are historically fragmented Mediterranean habitats and settings for the evolution of many endangered soil-specialist plants with narrow ranges.

- In this study, we aimed to determine how fragmentation (area and connectivity) affects: (i) pollinator community composition and (ii) structural properties of pollination networks; and whether there are differences in the effects of fragmentation on: (iii) the number of interactions and visits among pollinator functional groups; and (iv) the number of interactions and specialisation degree between soil-specialist and soil-generalist plants. We characterised the degree of fragmentation and the pollination network structures in 12 gypsum habitat fragments embedded in a cropland matrix during two consecutive years.

- We found significant relationships between fragmentation and network structure. The effects of fragmentation differed among pollinator functional groups, but not between soil-specialist and soil-generalist plants, in terms of number of interactions. However, the relatively higher pollinator specialisation of soil-specialist plants suggested greater dependence on pollinators.

- Inter-annual variations in the network structures demonstrated the importance of temporal replication. The observed patterns related to the landscape structure and pollination at both the network and species levels provide insights into the key ecological processes in gypsum islands. These findings may help to identify the potential drivers of species persistence, especially for endangered soil-specialist plants with narrow ranges in a changing scenario with exacerbated habitat fragmentation.

INTRODUCTION

Habitat fragmentation is one of the most pervasive threats to biodiversity (Fahrig 2003; Collinge 2009; Hagen *et al.* 2012). Among its most immediate effects, population isolation has critical consequences for plant reproduction, which are usually mediated by drastic decreases in the quantity and quality of animal services (Eckert *et al.* 2010). In particular, fragmentation can modify plant–animal mutualistic relationships with both pollinators (Fægri & Pijl 1979; Aizen & Feinsinger 1994; Aguilar *et al.* 2006; Hagen *et al.* 2012) and dispersers (Howe 1984; Cordeiro & Howe 2001; Rodríguez-Cabal *et al.* 2007; McConkey *et al.* 2012). Animals that provide services become scarce in small and isolated habitat fragments partly because the reduced plant populations are less attractive to them (Kruess & Tscharntke 2000; Tewksbury *et al.* 2002; Townsend & Levey 2005; Kolb 2008). The frequency and effectiveness of interactions may be reduced by changes in the identity of interacting species or their degree of specialisation (Burkle & Knight 2012).

From the perspective of conservation, there is no doubt that species diversity and so-called interaction diversity should both

be conserved (Ehrlich & Raven 1964; Bond 1994; Kearns *et al.* 1998; Tylianakis *et al.* 2010; Valiente-Banuet *et al.* 2014). Interactions between plants and pollinators do not occur between isolated pairs of species. In contrast a variable and complex network of interactions connects all species within plant and pollinator communities (Bascompte 2009). Changes in landscape structure may profoundly affect the structure and functioning of these interactions at the network level (Spiesman & Inouye 2013; Moreira *et al.* 2015). However, researchers have only recently begun to study the effects of habitat loss and fragmentation on pollination networks (Hagen & Kraemer 2010; Ferreira *et al.* 2013; Kormann *et al.* 2016). This is important because the extinction of interactions may occur prior to species extinctions due to habitat changes (Sabatino *et al.* 2010; Burkle & Knight 2012; Burkle *et al.* 2013; Evans *et al.* 2013; Valiente-Banuet *et al.* 2014), thereby providing a useful indicator of losses of functionality at the global ecosystem scale.

Previous studies suggest that decreases in the area and connectivity of the fragments may lead to losses of interaction diversity (Sabatino *et al.* 2010; Burkle & Knight 2012; Kormann *et al.* 2016). In smaller fragments, the number of pollination interactions and visits per species are lower (Aizen *et al.* 2012;

Burkle & Knight 2012), and specialist pollinators expand their resource niche (Burkle & Knight 2012). Habitat degradation may also affect the degree of specialisation of species (Nielsen & Totland 2014), reduce interaction diversity and increase the number of interactions per species and the tendency of networks to be structured into clusters (modules) of species that are densely connected among themselves (Spiesman & Inouye 2013). These previous results suggest important effects of habitat degradation, loss and fragmentation on pollination networks, but we still lack a complete understanding of the overall process (Astegiano et al. 2015).

Mediterranean ecosystems that have developed on gypsum soils are very interesting for investigating the effects of landscape structure on pollination networks (Escavy et al. 2012; Escudero et al. 2015). These ecosystems have a naturally fragmented spatial structure because the gypsum soil is immersed within other types of soil and geological substrates. The soil in these gypsum islands has a highly restrictive effect on plant development due to its physical and chemical properties (Escudero et al. 2015), thereby leading to unusual assemblies of plants, most of which are narrow endemic specialists, including a high percentage of threatened species (Martínez-Hernandez et al. 2011). These highly restrictive effects on plants also mean that most of these areas have not been exploited for agriculture, which has reinforced their island-like configuration. Understanding the structure of pollination networks in these island-like habitats may help to clarify the effects of habitat fragmentation on highly humanised landscapes such as the Mediterranean. Moreover, it would help to explain how fragmentation affects the interactions of soil-specialist plants and whether this adaptive edaphic shift has conditioned the ability of these species to build robust pollination networks.

Thus, in the present study, we aimed to determine how fragmentation (area and connectivity) might affect: (i) pollinator community composition and (ii) structural properties of pollination networks; and whether there are differences in the effects of fragmentation on: (iii) the number of interactions and visits among pollinator functional groups; and (iv) the number of interactions and specialisation degree between soil-specialist and soil-generalist plants. We hypothesised that fragmentation may affect both the pollinator composition and network structure. Larger and more connected fragments could have a higher number of species and interactions per species, thereby affecting the network dimensions, and a less random network assemblage, which might influence the second-order properties of the network. The effects of the area of fragments on the network properties will be modulated by the connectivity of fragments. Well-connected fragments might have more species and interactions than expected for a specific fragment area simply because of differences in the probability of colonisation (Giladi et al. 2011; Jones et al. 2015). In particular, habitat fragmentation would affect pollinators with reduced mobility. We also considered that soil-specialist plants, which have evolved in naturally fragmented habitats, would be affected less by fragmentation than soil-generalist plants. The results obtained in the present study may enable us to understand the effects of historical fragmentation and to predict the potential risk of subsequent fragmentation in this and other habitats. To the best of our knowledge, this is also the first time that temporal replication (two consecutive years) has been employed in an analysis of the effects of habitat fragmentation on pollination networks, which is especially important for highly stochastic climates such as that in the Mediterranean.

MATERIAL AND METHODS

Study area

This study was conducted in a fragmented gypsum landscape in central Spain, where the fragments are immersed in a cropland matrix (Fig. 1). The main crops in the area are dryland cereals. This area has a semi-arid Mediterranean climate, with a mean annual rainfall of 525 mm·m^{-2}·year^{-1} and average annual temperature of 13.7 °C (at the nearest weather station in Belinchón, Cuenca 30T 495735X; 4433384Y). Most of the precipitation occurs during the early spring and late autumn, with extremely intense summer droughts. The soils are classified as typical Gypsiorthid and the gypsum content exceeds 80%. The vegetation comprises a gypsiferous shrubby community dominated by soil-specialist species such as *Helianthemum squamatum* (L.) Dum.-Cours., *Lepidium subulatum* L. and some gypsovags, which are also able to occur in other types of soil, *e.g.* the tussock *Stipa tenacissima* L. (Table S1). The mean perennial plant cover is 36% (between 10% and 68%) and open areas have a well-developed biological soil crust (BSC) dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (G. H. Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt and *Psora decipiens* (Hedw.) Hoffm. In these open areas, the BSCs coexist with a highly diverse plant community of tiny annual ephemerals, which comprise a regional species pool of nearly 120 species and up to 30 species 0.25 m^{-2} in rainy years (Luzuriaga et al. 2012, 2015).

In a parallel study, we analysed the effects of fragmentation on plant diversity by sampling 50 habitat fragments selected at random over a rectangular surface of 3 × 13 km (Luzuriaga et al. in preparation). We haphazardly selected 12 of these habitat fragments covering the range of fragment area sizes and connectivity in this area. To avoid edge bias, this subset only included fragments located 500 m away from the sampled perimeter.

Fragment characterisation

We calculated two parameters to describe the landscape structure: fragment area and connectivity (Table S2). Fragment area was measured using aerial photographs and *Conefor* software (Saura & Torné 2009). To estimate the fragment connectivity, a larger quadrat measuring 13 × 6 km was employed after determining the sizes of all the fragments (Fig. 1). The connectivity of each fragment was then quantified using a proximity index, which considered the number of surrounding fragments weighted by their distance to the target fragment (Tremlová & Münzbergová 2007; Matesanz et al. 2015):

$$C_j = \log 10 \sum\nolimits_{\{z=1\}}^{\{n\}} A_z / d_{jz}^2$$

$j \neq z$, where C_j is the connectivity of fragment j, z is the total number of fragments around target fragment j that are within a 500-m buffer zone, A_z is the area of fragment z, and d_{jz} is the
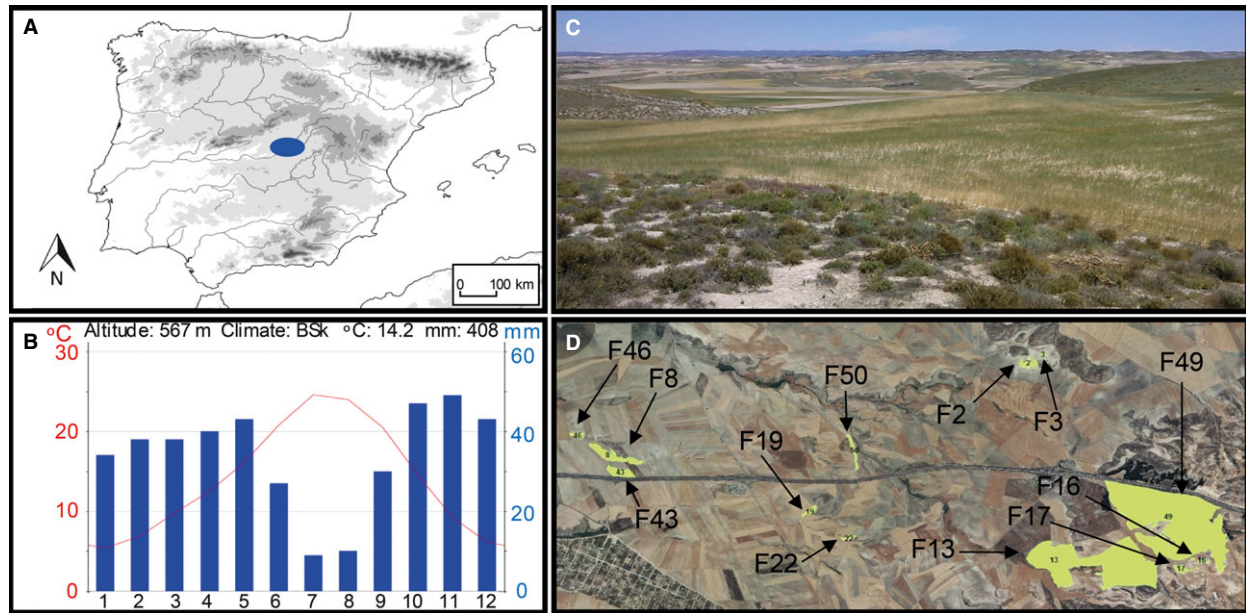
Fig. 1. Study area: A: map showing the geographic locations of the gypsum outcrops investigated in Central Spain. B: Climograph with data for the mean annual temperature and total annual precipitation. C: Image of the gypsum outcrop landscape. D: Aerial photograph of the study area showing the spatial distribution of habitat fragments distributed in the cropland matrix.

minimum edge-to-edge distance between fragment j and z. Negative values of C_j were set to zero.

Interaction sampling

Plant–pollinator interactions were sampled at 12 selected habitat fragments in two consecutive years (2013 and 2014) during May and June (when most of the plant species in the study area were at their flowering peak), which covered almost the whole diurnal period of flower visitor activity (from 10:00 to 17:00 h). On every sunny and windless day, three people separately sampled six of the 12 fragments (two fragments per day and observer), so a full fragment rotation was completed every 2 days. The order of fragments in this rotation was first randomised and then corrected slightly until the time intervals and the observers were balanced among fragments. Sampling followed a nonlinear transect (approximately 150 min) covering all vegetation patches within the fragment, where the sampling effort was proportional to the abundance of flowers. All contacts (visits) observed at both sites (approximately 2.5 m either side) and ahead of the observer were recorded. We considered that a visit occurred only when the flower visitor ('pollinator' in the following) clearly touched the anthers and/or stigma of a flower. In the field, pollinators were classified as morphospecies according to their size, shape and colour pattern. Voucher morphospecies were captured and sent to specialists for identification (see the Acknowledgments) and they were deposited in the collection of the Biodiversity and Conservation Unit at Rey Juan Carlos University. The total sampling effort comprised 8488 visits in 2013 and 7701 visits in 2014 (481–1434 visits per fragment in 2013, and 321–1063 visits per fragment in 2014), with a total of 182.6 h in 2013 and 196.8 h in 2014 (11.7–21.2 h per fragment in 2013, and 13.8–19.5 h per fragment in 2014). All of the fragments were (based on

rarefaction curves and Chao1 estimator) sampled equally (Tables S3, S4, Figures S1, S2).

Pollinator functional groups

We allocated pollinator species to 'pollinator functional groups' (*sensu* Fenster *et al.* 2004), according to the criteria given in Gómez *et al.* (2008), *i.e.* similarity in body size, length of the proboscis and foraging habits. We identified 26 functional groups: (i) Ephemeroptera, (ii) Hemiptera, (iii) Neuroptera, (iv) butterflies, (v) diurnal moths, (vi) Sphingidae, (vii) Sesiidae, (viii) bee flies (long-tongued nemestrinid and bombilid flies), (ix) hoverflies (syrphid flies and short-tongued bombilid flies), (x) big flies (non-syrphid flies with a body size similar or larger than *Musca domestica* Linnaeus, 1758), (xi) small flies (non-syrphid flies with a body size smaller than *M. domestica*), (xii) Symphyta, (xiii) Mutillidae, (xiv) ants, (xv) big sphecoids (big Sphecidae and Crabronidae), (xvi) sphecoids (small Sphecidae and Crabronidae), (xvii) Chrysididae, (xviii) wasps (Braconidae, Ichneumonidae, Pompilidae, Thiphiidae and Vespidae), (xix) big bees (bees with a body size larger than *Apis mellifera* L., 1758 but smaller than *Xylocopa violacea* (L., 1758), (xx) medium-sized bees (with a body size similar to *Anthidium cingulatum* Latreille, 1809), (xxi) small bees (with a body size similar to *Ceratina cucurbitina* Rossi, 1792), (xxii) tiny bees (*Nomioides minutissimus* Rossi, 1790), (xxiii) *Xylocopa violacea*, (xxiv) big beetles (non-curculionid Coleoptera with a body size >2 mm), (xxv) small beetles (≤2 mm) and (xxvi) Curculionidae.

Network structural properties

A quantitative pollination network was built for each fragment and year, and for each network, we used the bipartite package

(Dormann *et al.* 2008) in R 3.3.1 (R Core Team 2016) to calculate the following network structural properties (Table S5).

Number of plants (V): plant species that received pollination visits.

Number of pollinators (A): pollinator species that visited flowers.

Number of interactions (L): contacts recorded between plant and pollinator species.

Interaction diversity: Shannon diversity of interactions (instead of species).

Interaction evenness: Shannon evenness of interactions.

Interaction dominance: dominance of interactions estimated as the fraction of the total interactions corresponding to the most frequently observed interaction minus 1/L (*sensu* Sabatino *et al.* 2010).

Connectance (C): fraction of actual interactions relative to all possible interactions calculated as $C = L/A \times P$ (Jordano 1987). Higher connectance suggests greater robustness to species extinction due to a higher number of interactions per species (Santamaría *et al.* 2014).

Web asymmetry (WA): ratio of pollinator (A) relative to plant (P) species calculated as $WA = (A-P)/(P + A)$. A positive value for WA indicates more pollinator species and a negative value for WA indicates more plant species. WA determines the number of potential partners per species of plant and pollinator. Hence, a higher value for WA suggests the greater robustness of plants to pollinator extinction (Santamaría *et al.* 2014).

Modularity: the extent to which the network is organised into clusters or modules of species that interact significantly more within their module than between modules (Olesen *et al.* 2007). We determined modularity using the QuanBiMo algorithm proposed by Dormann & Strauss (2014), which is the most suitable alternative for analysing modularity for pollination networks because it uses bipartite quantitative networks. This method uses a recurrent Markov chain Monte Carlo (MCMC) algorithm to find the best configuration of modules. To obtain reliable modularity values for the 24 networks, we used 10^8 MCMC steps with a tolerance level of 10^{-10} (Dormann & Strauss 2014).

Nestedness: the extent to which specialised species tend to interact with appropriate subsets of the species that interact with more generalised species (Bascompte *et al.* 2003). Therefore, in a nested network, a core of generalist species interacts among themselves providing robustness to the whole network, and specialist species interact with generalist species but not among themselves. We used the index proposed by Almeida-Neto & Ulrich (2011), which is the most suitable for analysing the nestedness of pollination networks because it uses quantitative networks. This index ranges between 0 and 100 representing non-nestedness and perfect nestedness, respectively.

Specialisation (H_2'): network-level specialisation of all interacting species of plants and pollinators based on the Shannon entropy, which removes the effect of marginal totals of species interactions. This index ranges between 0 and 1 representing extreme generalisation and specialisation, respectively (Blüthgen *et al.* 2006). In general, species are more selective when choosing their mutualistic partners in a specialised network.

These structural properties provide information about species richness (V and A), interaction richness and diversity (L, interaction diversity, interaction evenness, interaction dominance and C), network properties related to robustness (C,

WA, nestedness and modularity) and the degree of specialisation and niche partition in the network (H_2').

Data analysis

We used generalised linear models (GLMs) to assess the effects of the landscape structure (fragment area and connectivity) on the structural properties of the 24 networks. The effects of fragment area and connectivity were also tested on: (i) the number of interactions and (ii) the number of visits observed separately for each of the pollinator functional groups (n = 26). Year was included as a repeated measure factor in all the models. The error distribution of each response variable was checked to select the appropriate link function. We used a quasi-likelihood algorithm in all the GLMs. The maximum-likelihood method was used for estimating parameters and Wald χ^2-tests were used to assess the significance of predictors.

Finally, we evaluated the effects of fragment structure on: (i) the number of visits and (ii) the degree of pollinator specialisation on plants (d'; Blüthgen *et al.* 2006) between soil specialists and soil generalists for each year. Soil-specialist species included true soil-specialist species or gypsophiles and soil-tolerant species or gypsovags *sensu* Palacio *et al.* (2014), whereas soil-generalist (non-specialist) species included species that also occurred on other substrate types (Table S1). The specialisation index d' represents the extent to which a species deviates from random sampling of the available interacting partners (Blüthgen *et al.* 2006), where it ranges from 0 (no specialisation) to 1 (perfect specialisation). We performed two separate two-way generalised linear mixed models (GLMM), and in both cases, we considered the fixed-effect factors of soil specialisation (soil-specialist *versus* soil-generalist plant) and year, and the random-effect factors of fragment and species. We employed a Poisson error distribution and log link function for the first model, and a binomial error distribution and logit link function for the second model. Significant differences in the average number of visits or degree of pollinator specialisation across plant groups were determined using an *a posteriori* Tukey's honest significant differences test. Statistical analyses were performed using SPSS version 15 (SPSS, Chicago, IL, USA) and the lme4 package (Bates *et al.* 2015) in R 3.3.1 (R Core Team 2016).

RESULTS

Composition of the pollinator community

In both years, big beetles visited more than 65% of plants and small beetles visited more than 50% of plants, which together accounted for 60% of the recorded visits (Fig. 2). This clear dominance of beetles was observed in all fragments and was consistent between years (Figures S2, S3, S4, S5, S6), where 10–35% of plants were visited by big beetles (7–66% of visits), followed by 7–27% of plants visited by small beetles (7–57% of visits), 4–27% of plants visited by bee flies (1–33% of visits) and 2–20% of plants visited by small bees (0–10% of visits).

Effects of fragmentation on the network structure

An increase in fragment connectivity was significantly related to an increase in the number of visited plants and thus a
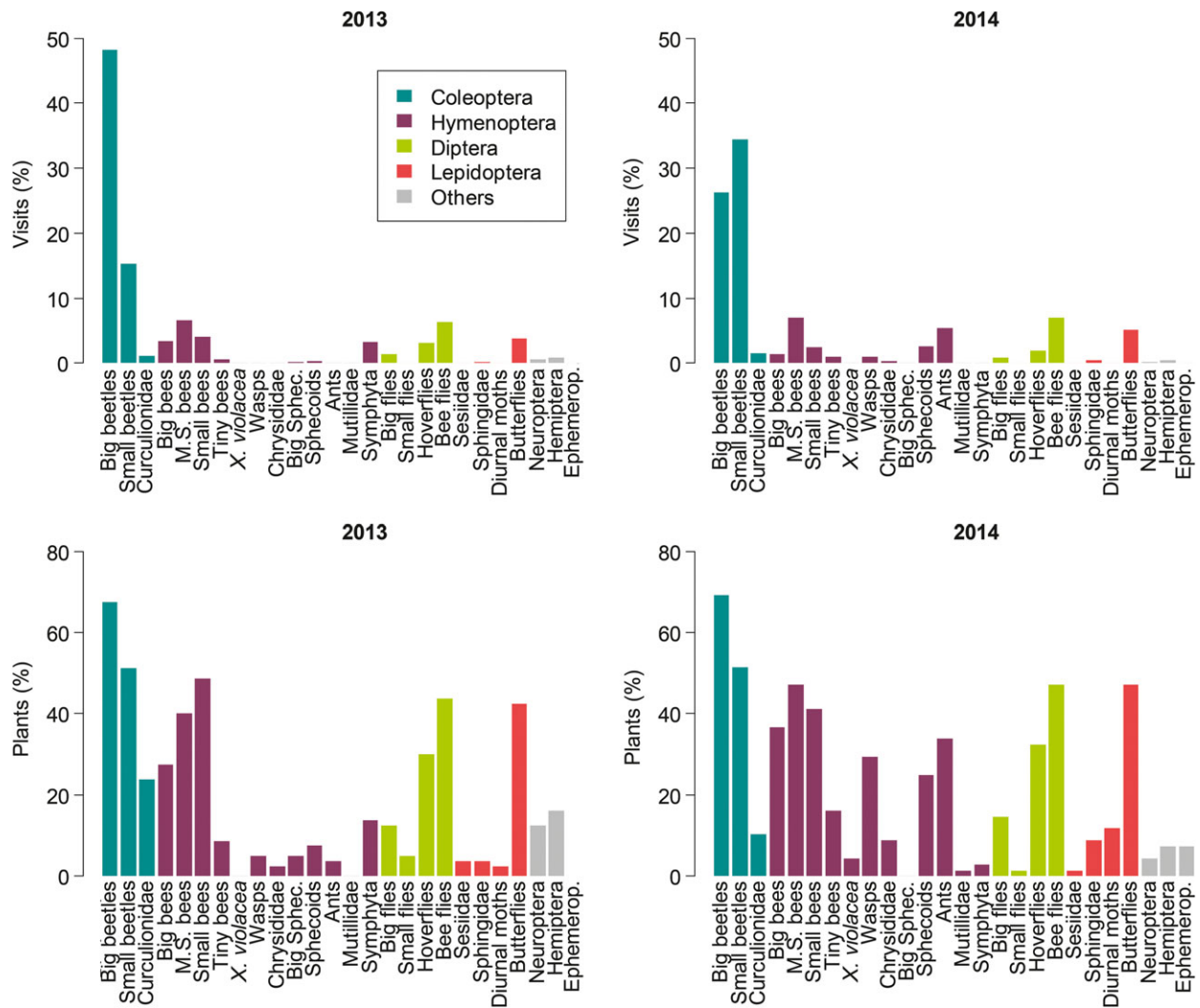
Fig. 2. Percentage of visits (upper panels) and percentage of plant species visited (lower panels) by each pollinator functional group during 2013 (left) and 2014 (right).

decrease in WA (Fig. 3), while an increase in fragment area was significantly related to increases in the number of visits and number of pollinators, and thus WA (Fig. 4). Interaction diversity and evenness increased significantly with fragment connectivity (Fig. 3). Modularity also increased significantly with fragment area (Fig. 4) and decreased as the fragment connectivity increased (Fig. 3). Nestedness decreased significantly with increased connectivity (Fig. 3). We found no other significant relationships (Table 1). Both the network dimensions and second-order structural properties, such as the network-level specialisation of interactions and nestedness, exhibited significant differences between years (Table 1). The remaining network variables remained unchanged between years (Table 1).

Effects of fragmentation on pollinator functional groups

The effects of landscape structure differed among the functional groups of pollinators (Table 2). Again, we determined a high rate of inter-annual variability, where 16 out of 26 functional groups differed significantly in terms of their number of

interactions and/or visits between years. Half of these groups had a higher number of interactions and/or visits in 2013, whereas the others exhibited the opposite pattern. In terms of the landscape structure, most of the observed relationships were related to fragment connectivity. Seven (bee flies, big bees, Ephemeroptera, Neuroptera, small beetles, small flies and tiny bees) of 26 functional groups participated in a lower number of interactions and/or visits in these less connected habitat fragments. Only sphecoids were negatively affected by fragment connectivity, and this was also the only group with a higher number of interactions in bigger habitat fragments.

Effects of fragmentation on soil-specialist *versus* soil-generalist plants

The GLMM detected significant effects on the number of pollination visits of soil specialisation, year and their interaction, but not of area or connectivity. In 2013, soil-specialist plants were visited significantly more than generalists, but this difference was not significant in 2014 (Fig. 5). Soil specialists
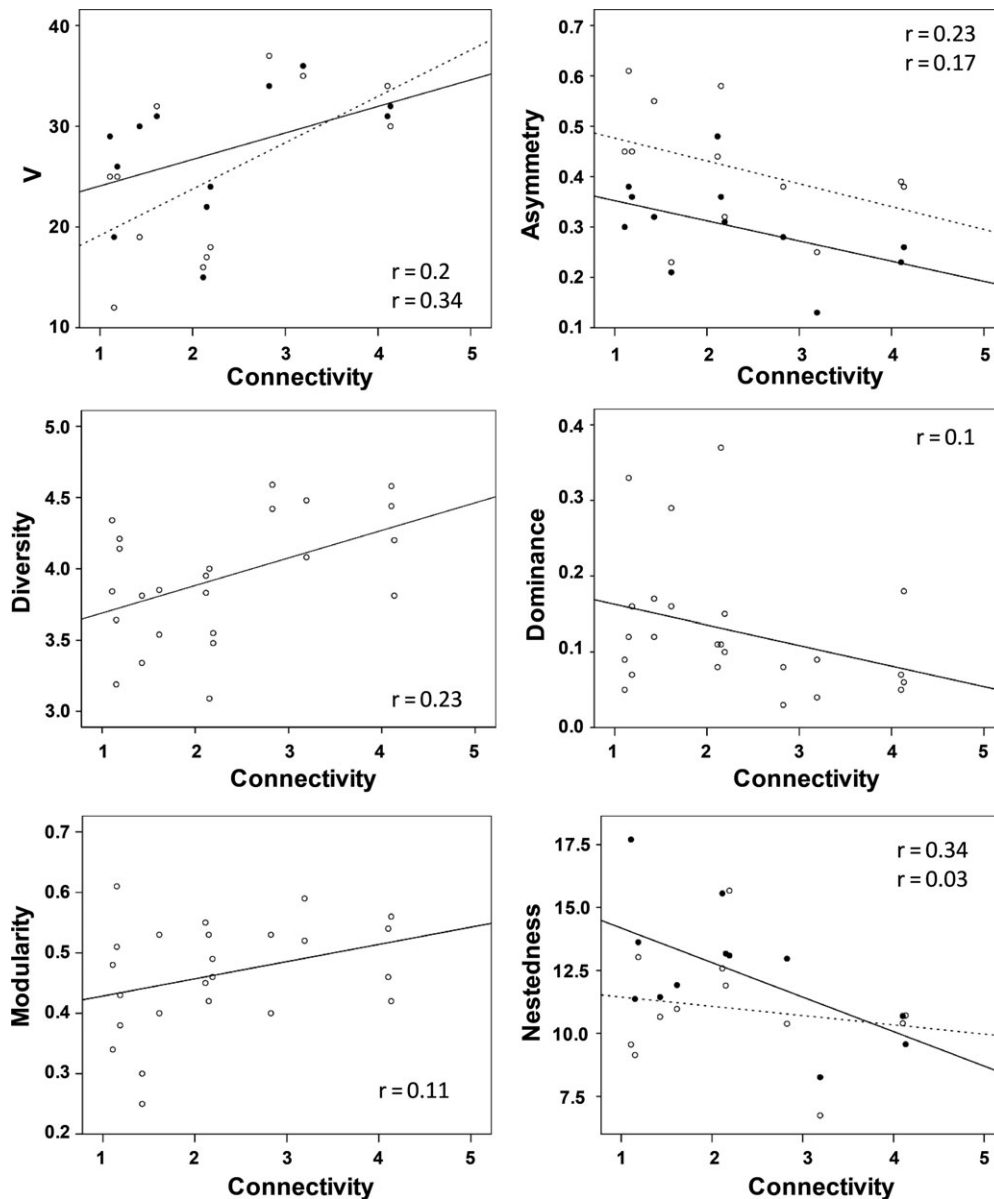
Fig. 3. Scatter plots showing the relationship between network properties and fragment connectivity. Coefficients of the linear correlation (r) are shown. In those variables showing significant differences between years, the closed circles and continuous lines correspond to 2013, and the open circles and dashed lines correspond to 2014.

received more visits in 2014 than 2013, whereas soil generalists received more visits in 2013 than 2014 (Fig. 6). Plant specialisation for pollination was significantly affected by: (i) soil specialisation, (ii) year and (iii) their interaction, but not by area or connectivity. Soil-specialist plants were more pollinator-specialised than soil-generalist plants in both years. In addition, both plant groups were more pollinator-specialised in 2014 than 2013 (Fig. 6).

DISCUSSION

In this study, we found that the landscape structure and pollination network configuration of the gypsum soil islands appeared to be tightly connected. In particular, fragmentation was related to the richness of plant and pollinator

species, the frequency and diversity of pollination interactions and the topology (nestedness and modularity) of the networks. In addition, we found inter-annual variations in the network properties for species richness and number of interactions, as well as for second-order properties such as the nestedness and network-level specialisation of interactions. We also note that the effects of landscape structure on the number of pollination interactions and visits differed among pollinator functional groups, where most were positively related to habitat connectivity. However, we detected no differences between soil-specialist and soil-generalist plants, although soil-specialist plants were visited more frequently than soil-generalist plants in 2013 and were more selective in their interactions with pollinator species in both years.
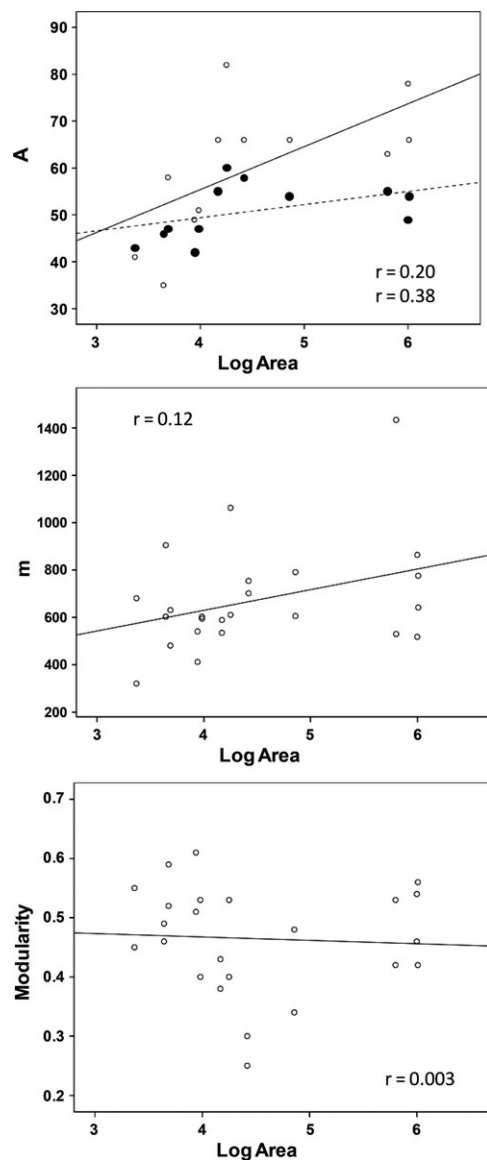
Fig. 4. Scatter plots showing the relationship between network properties and fragment area. Coefficients of the linear correlation (r) are shown. In those variables showing significant differences between years, closed circles and continuous lines correspond to 2013, and open circles and dashed lines correspond to 2014.

Composition of the pollinator community and network structure of gypsum islands

Plants inhabiting gypsum islands were visited by a rich pollinator community comprising 26 functional groups, which corresponded to seven orders of insects. The dominance of beetles is consistent with the results of numerous previous studies that linked pollination by beetles with Mediterranean and semi-arid climates (*e.g.* Grant 1950; Dafni *et al.* 1990; Martínez-Harms *et al.* 2012; Karolyi *et al.* 2016). Compared with the 52 pollination networks analysed by Traveset *et al.* (2016), our 24 networks in gypsum islands had intermediate values for interaction diversity, which may have been due partly to their intermediate species richness values. The connectance and nestedness values were moderate with a

relatively broad range of variation (Traveset *et al.* 2016). In contrast to expectations for genuine arid environments (Trøjelsgaard & Olesen 2013), the modularity was relatively high for all networks in both years compared with the 22 networks analysed by Dormann & Strauss (2014). However, the network-level degree of specialisation covered the entire range of values recorded previously (Blüthgen *et al.* 2007; Traveset *et al.* 2016). The structural characteristics of these networks were within the broad range represented by other Mediterranean pollination networks (Traveset *et al.* 2016). However, the lack of quantitative analyses of bipartite modularity in other Mediterranean networks prevented definitive comparisons.

Effects of fragmentation on the network structure

The landscape structure was related to the species richness, interaction frequency and diversity, and network configuration in a complex manner. Previous studies found relationships between both connectivity and fragment size with interaction diversity (Sabatino *et al.* 2010; Burkle & Knight 2012; Kormann *et al.* 2016), but we only detected a relationship between fragment connectivity and interaction diversity. Fragment connectivity was also related to plant richness but not to pollinator richness. Pollinator richness increased with the fragment size, probably due to the well-known species–area relationship. Small-scale environmental changes are important for the foraging decisions of pollinators (Geslin *et al.* 2013; Seifan *et al.* 2014; Torné-Noguera *et al.* 2014), so this finding could be related to the fact that larger fragments have higher small-scale environmental heterogeneity. Indeed, fragment size did influence the frequency of pollination visits, which reinforces the relationship between fragment size and pollination function, as reported previously (Aizen *et al.* 2012; Burkle & Knight 2012). The fragment connectivity–modularity and fragment area–modularity relationships were in opposite directions, and the same applied to the fragment connectivity–modularity and fragment connectivity–nestedness relationships. Overall, these results and the high inter-annual variations in network metrics indicate that the effects of landscape structure on the network topology are very difficult to predict.

There is a consensus that modularity is the result of the combination of species phylogenies and the convergence of traits (Olesen *et al.* 2007; Donatti *et al.* 2011; Danieli-Silva *et al.* 2012). The positive relationship between fragment connectivity and modularity suggests lower specialisation in pollination interactions at the species group level and higher randomness of the network construction in the most isolated fragments (Olesen *et al.* 2007). The negative relationship between fragment size and modularity agrees with previous findings (Spiesman & Inouye 2013). The higher modularity in smaller fragments may have been related to the local extinction of generalist species. Furthermore, lower modularity has been linked to the higher stability of pollination networks under simulated population dynamics (Thébault & Fontaine 2010), so networks belonging to smaller fragments could be less stable. However, the negative relationship between fragment connectivity and nestedness is consistent with the idea that less diverse and specialised interactions prevail in more isolated fragments.

Table 1. Effects of year (2013 or 2014), area (log transformed) and connectivity on the observed network descriptors.

	V	A	WA	L	m	interaction diversity	interaction evenness	interaction dominance	C	H$_2$′	modularity	nestedness
(Intercept)	***	***	***	***	***	***	***	***	***	***	***	***
Year	*	***	***							**		*
Log area		(+)***			(+)*						(−)*	
Connectivity	(+)*		(−)***			(+)*	(+)*	(−)*			(+)*	(−)*

Asterisks show the significant level (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) associated with Type III Wald chi-squared values obtained from GLMs, with year as the within subject factor and log area and connectivity as co-variables. Normal distribution and identity link function were used in all models, except for number of plants (P), number of pollinators (A), Web Asymmetry (WA), number of interactions (L), number of visits (m) and Connectance (C) models, which were better adjusted using gamma distribution and log link function. The sign of each significant relationship is shown in parentheses.

Table 2. Effects of year, fragment area (log-transformed) and connectivity on the number of interactions (L) and visits (m) observed for different functional groups of pollinators.

	year		log area		connectivity	
functional group	L	m	L	m	L	m
Ants	***(2014)	***(2014)				
Bee flies		***(2014)			***(+)	
Big bees		***(2014)			***(+)	
Big beetles	***(2013)	*(2013)				
Big flies						
Big sphecoids	***(2013)	*(2013)				
Butterflies						
Chrysididae	***(2014)	*(2014)				
Curculionidae	***(2013)					
Diurnal moths						
Ephemeroptera	*(2014)	*(2014)	0.06 (−)		*(+)	
Hemiptera	*(2013)					
Hoverflies		**(2013)				
Medium-sized bees						
Mutillidae						
Neuroptera	*(2013)				**(+)	
Sesiidae						
Small bees	***(2013)	**(2013)				
Small beetles		***(2014)			***(+)	
Small flies					*(+)	0.07(+)
Sphecoids	***(2014)	***(2014)	***(+)		**(−)	0.059(−)
Sphingidae						
Symphyta	***(2013)					
Tiny bees					**(+)	
Wasps	***(2014)	***(2014)				
Xylocopa violacea						

Asterisks show the significant level (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) associated with Type III Wald Chi-squared values obtained from GLMs, with year as the within subject factor and log area and connectivity as co-variables. Normal distribution and identity link function were employed in all models before log transformation of the number of interactions (small beetles) and visits (by ants, bee flies, big bees, big flies, butterflies, Curculionidae, Hemiptera, medium bees, Neuroptera, small bees, small beetles, Symphyta, and tiny bees). The year with a higher number of observations and the sign of the relationship are shown in parentheses.

Effects of fragmentation on pollinator functional groups

Some significant differences were found when we analysed the information separately for the different pollinator functional groups relative to the landscape configuration. Important pollinator groups in gypsum islands in terms of the number of visits and number of visited plants, such as small beetles or big and tiny bees, exhibited abrupt decreases in more isolated fragments, whereas other groups such as medium-sized bees and big beetles appeared to move easily and without limitation among the fragments. This critical isolation sensitivity of small beetles and tiny bees may have been related to their reduced flight capacity. Tiny insects would have difficulty reaching neighbouring gypsum remnants, although the maintenance of pollinator richness according to all our connectivity values suggests that the surrounding crops did not represent a harsh matrix for most pollinators. Therefore, it is necessary to consider whether human-driven fragmentation might increase due to cropland intensification, because the observed reductions in the number of interactions and visits in some groups will
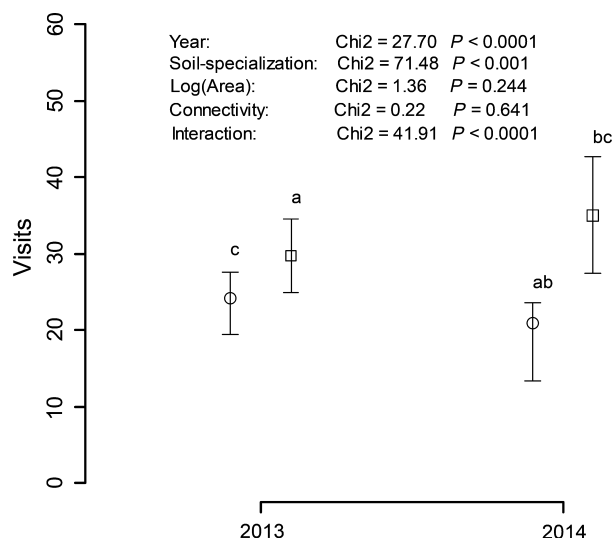
Fig. 5. Mean number of pollination visits to generalist plants (circles) and gypsum-specialist plants (squares) in 2013 (left) and in 2014 (right). N = 333 in 2013 and N = 300 in 2014. Error bars correspond to SE. Chi-squared (χ^2) and significance (*P*) according to two-way GLMM to compare the differences in the number of visits with soil specialisation and year as factors. Fragment and species were considered as random-effect factors. Average values with dissimilar letters differ significantly according to an *a posteriori* Tukey's honest significant differences test.
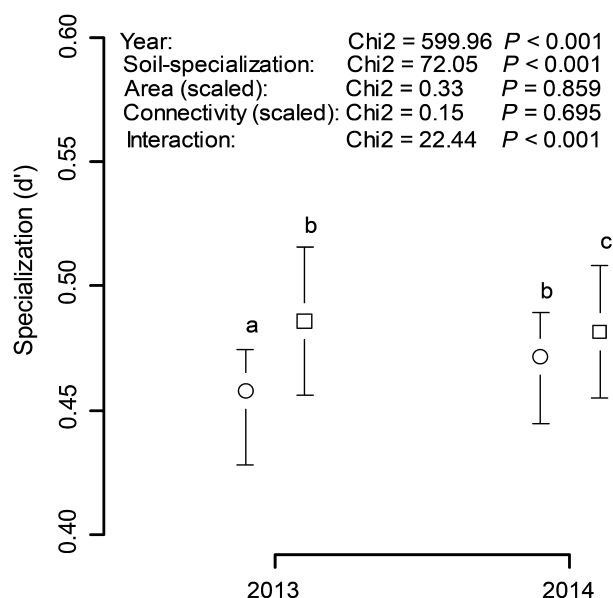
Fig. 6. Mean values for specialisation (d'; Blüthgen *et al.* 2006) on generalist plants (circles) and gypsum-specialist plants (squares) in 2013 (left) and in 2014 (right). N = 333 in 2013 and N = 300 in 2014. Error bars correspond to SE. Chi-squared (χ^2) and significance (*P*) according to a two-way GLM to compare differences in the number of visits with soil specialisation and year as factors. Fragment and species were considered as random-effect factors. Average values with dissimilar letters differ significantly according to an *a posteriori* Tukey's honest significant differences test.

ultimately lead to reductions in richness and loss of interactions. Finally, inter-annual variations in insect abundances due to changes in weather conditions might partly explain some of

the differences between years in terms of the effects of fragmentation on the participation of each pollinator functional group.

Effects of fragmentation on soil-specialist *versus* soil-generalist plants

It would seem that no advantage was conferred on soil-specialist plants over generalist plants under fragmentation in terms of pollen availability, despite their evolution in an insular environment comprising gypsum outcrops in a matrix of other type of soils. The higher number of pollinator visits to soil-specialist plants compared with soil-generalist plants during 2013 suggests an important functional role for soil-specialist plants in the pollination network. Furthermore, the higher value of the d' index for soil-specialist species compared with generalist species in both years may indicate that soil specialisation has been accompanied by a relative specialisation strategy by plants on their pollinators. A relative preference of pollinators on these gypsum specialists could be explained partly by the relative abundances of species (Stang *et al.* 2006; Vázquez *et al.* 2007). However, this was not the case for the differences in specialisation for pollination interactions because the d' index indicated higher proportional use compared with availability (Blüthgen *et al.* 2006). The higher specialisation in terms of their interaction partners by soil-specialist plants suggests greater vulnerability because they might depend on a more specific set of pollinator species (Bond 1994).

CONCLUSIONS

In this study, we showed that the pollination networks on gypsum islands, which were characterised by high functional richness of pollinator species, dominance by beetles, moderate connectance and nestedness, and high modularity, were related to the landscape spatial structure. Plant richness was positively related to fragment connectivity, whereas pollinator richness and frequency of visits were positively related to fragment size. Year and fragment connectivity partly explained the effects of fragmentation on the participation of different functional groups of pollinators in the networks. The relationship between fragmentation and modularity suggests higher randomness in the network configuration of the most isolated fragments and potentially lower stability of the network for the smaller fragments. It appeared that no advantage was conferred on soil-specialist plants compared with soil-generalist plants under fragmentation in terms of pollen availability, despite their evolution in the context of landscape fragmentation. Soil specialisation by plants appears to have led to relative specialisation on their pollinators, which could make them more vulnerable to future changes in the composition of pollinators. The observed inter-annual variations in both the participation of pollinator functional groups and the network structure highlight the need for temporal replication in pollination network studies. In addition, combining network-level and species-level information can help to explain the complex effects of landscape structure on the pollination interactions on gypsum islands, as well as providing insights into previously unknown aspects of the reproductive biology of plants that live in these environments. This information is essential for understanding the potential determinants that drive the persistence of the numerous endangered soil-specialist plants with narrow ranges.

Especially, in a changing scenario where habitat fragmentation will be exacerbated (Matesanz *et al.* 2009), as well as the effects of other drivers of global change such as warming (Sánchez *et al.* 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Plant species included in the plant–pollinator networks classified according to their specificity to gypsum soils (see Material and Methods for further details of this classification).

Table S2. Area and connectivity of the 12 fragments investigated in the study. See the Material and Methods for details of the calculation of connectivity.

Table S3. Estimated sampling completeness for each fragment in 2013. Percentage of sampled interactions corresponding to the asymptotes of record-based rarefaction curves and the Chao1 estimator (Gotelli & Colwell 2010).

Table S4. Estimated sampling completeness for each fragment in 2014 (see Table S1 for details).

Table S5. Network structural properties corresponding to the 12 fragments in two consecutive years.

Figure S1. Rarefactions curves for the estimated interaction richness *versus* increasing number of records (visits) and 95% confidence intervals (dotted line) for each fragment in 2013 (see Table S1 for details).

Figure S2. Rarefactions curves for the estimated interaction richness *versus* increasing number of records (visits) and 95% confidence intervals (dotted line) for each fragment in 2014 (see Table S1 for details).

Figure S3. Percentage of visits corresponding to each pollinator functional group by fragment in 2013.

Figure S4. Percentage of visits corresponding to each pollinator functional group by fragment in 2014.

Figure S5. Percentage of plants visited by each pollinator functional group by fragment in 2013.

Figure S6. Percentage of plants visited by each pollinator functional group by fragment in 2014.

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