Delimiting plant diversity that is functionally related via interactions with diurnal pollinators: An expanded use of rarefaction curves☆

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ABSTRACT

The way in which taxonomic diversity relates to functional diversity is important for understanding the mechanisms that sustain ecosystem function and services. We investigated how an explicit consideration of plant-pollinator interactions influences our view of plant diversity. We studied three plant species-rich communities located in different biodiversity hotspot regions: two soil-specific plant communities with Mediterranean-climate, sandy dolomite outcrops in Andalusia (Spain) and serpentine seeps in California (USA), and a third community in the sub-tropical dry scrublands in Yucatan (Mexico). Sampling at three spatial scales (region, site, plot) and rarefaction analyses were used to characterize and compare spatial and temporal variation of entomophilous plant diversity based on species presence ("static plant diversity"), flower display sizes along the season ("dynamic flower diversity"), and pollinator visitation ("interaction-effective diversity"). The studied communities differed in the static diversity of plants, with sub-tropical dry scrublands being less diverse than the two Mediterranean communities. Reduction of static diversity at local scale was stronger in the richest Mediterranean communities and, thus, static diversity was similar among regions when considering finer (site-level) spatial scales. In addition, the two Mediterranean communities displayed more seasonal variation, thus reducing differences in dynamic diversity among regions, i.e. when considering finer temporal scales. These results suggested that, at finer spatio-temporal scales expected to be relevant for interactions with pollinators, plant communities are not necessarily as diverse as the region where they occur. Accordingly, interaction-effective diversity based on pollinator visitation was in all cases lower than expected relative to the diversity of flowers. Thus, diversity of visited flowers does not perfectly track diversity of flowering species but instead reaches asymptotes at much lower values than expected in the richest communities. Regional species diversity may support the functionality of interactions at broader spatial and temporal scales than they actually occur.

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1. Introduction

Understanding the mechanisms that promote, structure or endanger diversity and ecosystem services is valuable from fundamental and applied perspectives (Kreft and Jetz, 2007; Bellard et al., 2012; Chase, 2012). Long-term historical, biogeographical, and climatic factors contribute to explain broad-scale geographic patterns of species diversity, whereas, at the local community scale, contemporary ecological filters related to resource availability and species partitioning of such resources may influence community function by limiting the number of species that can coexist at a given site (Kreft and Jetz, 2007; Chase, 2012). Within local communities, there is growing consensus that local effects of diversity on ecosystem processes are attributable to functional traits of individual species and their interactions (how they compete directly or indirectly, and how they modify each other's biotic and abiotic environment) rather than to species number per se (Díaz and Cabido, 2001; Funk et al., 2016). Therefore, several authors have argued that

to be able to understand and preserve the complexity of ecosystem dynamics and services we need to understand community structure and realized interactions (Kareiva and Marvier, 2003; Hooper et al., 2005; Srivastava and Velend, 2005; Isbell et al., 2011).

Plant functional traits have proven powerful in explaining variance in community structure and ecological processes along environmental gradients (Funk et al., 2016 and references therein). In contrast, the assessment and comparison of the diversity of interactions between species at different trophic levels and their impact in community structure remain understudied (Burkle et al., 2016). One approach to fill this gap is to assess whether diversity varies in space and time within a single trophic level, and how this variation influences diversity in associated multi-trophic interactions. For instance, diversity estimates that are based on plant species occurrence or individual abundance provide a taxonomic *static* view of diversity relevant for understanding long-term plant–plant interactions such as spatial competition, facilitation or habitat partitioning (e.g. Cardelús et al., 2006; Brooker et al., 2008; Wilson et al., 2012; Michalet et al., 2015). However, describing diversity for multitrophic plant-animal interactions requires other descriptors that are capable of capturing the *dynamic* nature of such interactions, based on the ephemeral nature of specific plant resources (e.g., flowers, fruits, seeds, young leaves). For example, in the particular case of plant-pollinator interactions such descriptors could incorporate aspects of floral display size and the degree of phenological overlap among species within the community (Potts et al., 2003; Forrest et al., 2010). It is also well-known that species composition of co-flowering assemblages changes temporally, but perhaps less well appreciated is the significance that such variability in floral resources at different time scales (from within day to among years) may have on interactions with pollinators and subsequently on pollination sufficiency of plant reproduction (Fontaine et al., 2006; Hegland and Totland, 2008; Lázaro et al., 2009), i.e. on *interaction-effective* diversity and its outcome.

An approach that incorporates multiple community descriptors (e.g. static, dynamic, interaction-based) will not only capture a more refined measure of diversity, but will contribute to comprehending how plant taxonomic diversity is effectively related to plant-pollinator interactions (Burkle et al., 2016). Such understanding of plant-pollinator interactions at the community level is required to distinguish community-specific patterns (e.g., McCall and Primack, 1992), to appreciate the reproductive consequences of pollinator sharing (Mitchell et al., 2009; Alonso et al., 2010; Arceo-Gómez and Ashman, 2011; Arceo-Gómez et al., 2016), to infer pollinator-mediated floral evolution (Armbruster and Muchhala, 2009), and to preserve pollination services in both natural and managed ecosystems (Klein et al., 2009; Potts et al., 2010). This is, however, a difficult task due to intrinsic variability in species-specific pollinator assemblages (e.g., Herrera, 1988; Price et al., 2005; Ollerton et al., 2007) and the monumental effort required to accurately characterize pollinator assemblages when a high number of species flower simultaneously within a community (see e.g. Petanidou et al., 2008; Hegland et al., 2010; Fründ et al., 2011; Gibson et al., 2011; Chacoff et al., 2012).

In this study we sought to understand how much plant diversity is functionally related to interactions with pollinators? Towards this goal, we recorded species occurrence within permanent plots spatially replicated, and within them we monitored seasonally both flower availability and floral visits. Then, we implemented a rarefaction approach to analyze three views of plant diversity that should be relevant for plant-pollinator interactions: taxonomic (static), floral-based (dynamic), and pollinator visitation-based (interaction-effective). The rarefaction approach prevent erroneous interpretations in comparing interaction diversity among studies with unequal sample sizes (Gotelli and Colwell, 2001), easily arising in this context from changes in floral produc-

tion and visitation rates among study communities or periods (Blüthgen, 2010; see also Herrera, 2005). The study was conducted in three plant-species rich communities located in different biodiversity hotspot regions: two soil-specific plant communities with Mediterranean-climate, sandy dolomite outcrops in Andalusia (Spain) and serpentine seeps in California (USA), and a third community in the sub-tropical dry scrublands in Yucatan (Mexico). This large-scale comparison among distant regions will provide a more general view of how multitrophic interactions can influence diversity structure within highly diverse regions (Kareiva and Marvier, 2003; Hooper et al., 2005) where very little is known about structure and functionality, but where spatial and temporal variation in diversity is likely to be high (see e.g., Harrison, 1999; Freestone and Inouye, 2006; Blondel et al., 2010). Overall, in comparing static, dynamic and interaction-effective descriptors of diversity in contrasting plant communities, this study sheds light into the relevant local ecological filters influencing those ecological processes which necessarily involve multitrophic interactions.

2. Methods

2.1. Study sites

This study involved three geographically distant regions in Europe and North America (Table 1). In each region we focused on specific plant communities dominated by small woody or herbaceous perennials and annuals that are animal-pollinated and exhibit a marked flowering season. In California, we studied the flowering plant species found on serpentine seeps. These endemic-rich communities are adapted to serpentine soils and temporally variable water availability (Alexander et al., 2007; Freestone and Inouye, 2006). Here, we selected five serpentine seeps at the Donald and Sylvia McLaughlin Natural Reserve (northern California, USA) which were separated by 0.3–5 km linear distance within a grassland matrix. We observed plants and pollinators during the flowering season (June and July) in 2010.

In Andalusia, we studied the flowering plant species inhabiting sandy limestone-dolomitic outcrops of the Baetic Ranges, characterized by high species richness and endemism (Mota et al., 2008). Here, we studied five outcrops in the Natural Park of Cazorla, Segura y Las Villas (Jaén province, SE Spain), separated by 1.6–16 km linear distances in a heterogeneous, complex mountainous landscape. Observations were conducted during the flowering season (May and June) in 2010. At each site, the study was conducted within permanent exclosures to avoid the hazards of intense trampling and herbivory on marked plots, associated to high abundance of wild and domestic ungulates, and seasonal transit of domestic flocks (Cuartas and García-González, 1992).

In Mexico, we studied three sites representative of canopy clearings in the sub-tropical dry coastal scrublands located near the northern coast of the Yucatan which is the most plant endemic-rich area of the Yucatán Peninsula (Espadas Manrique et al., 2003). Study sites were separated by 0.5–5 km in a flat area and mostly surrounded by secondary vegetation growing on homogeneous limestone bedrock (karstic) soils (Duch, 1988). Here, observations were conducted during the flowering season associated to rains (July–September) in 2011.

2.2. Sampling scheme and data collection

In order to characterize the three plant communities in a comparable way, at each site we established permanent square plots every 5 m along linear transects (Table 1; see Appendix A in Supplementary material for a detailed description of the plot spatial layout and design features). We focused on the flowering com-

Table 1
Study sites location, number of plots sampled, average (± S.E.) number of species (sp) per plot and m², and the accumulated local species (sp) richness.

Region	Site (code)	Geographic location	No. plots	No. sp/plot[a]	No. sp/m²	Accumulated sp richness
Andalusia	Fuente Bermejo (F)	37°56′N/02°50′W	20	6.6 ± 0.3	1.65	14
	Arenales del Guadalentin (G)	37°55′N/02°50′W	24	9.7 ± 0.4	2.42	23
	Nava de las Correhuelas (N)	37°56′N/02°52′W	24	7.3 ± 0.4	1.82	19
	ca. Puerto Llano (P)	37°49′N/02°58′W	24	7.8 ± 0.3	1.95	13
	Raso del Madrigal (R)	37°55′N/02°53′W	20	9.3 ± 0.5	2.32	17
California	Banana Slug (BS)	38°52N/122°24′W	29	7.3 ± 0.3	3.65	20
	Research Hill A (RHA)	38°51′N/122°25′W	30	8.6 ± 0.5	4.30	21
	Research Hill B (RHB)	38°51′N/122°24′W	20	8.5 ± 0.6	4.25	18
	Tailings Pond 8 (TP8)	38°52′N/122°26′W	20	6.0 ± 0.4	3.00	13
	Tailings Pond West (TPW)	38°52′N/122°27′W	20	6.9 ± 0.4	3.45	20
Yucatan	Calvario (CAL)	21°15′N/89°35′W	20	6.1 ± 0.2	1.52	11
	Capilla (CAP)	21°17′N/89°35′W	20	5.8 ± 0.2	1.45	14
	Dzemul (DZE)	21°18′N/89°20′W	20	5.1 ± 0.4	1.27	12

[a] Plots were 2 × 2 m in Andalusia and Yucatan and 2 × 1 m in California, see Appendix A in Supplementary material for details.

munity of animal-pollinated plants characteristic of each habitat and therefore both wind-pollinated species (e.g., grasses, pines), as well as species that never flowered during the study period were not considered. Flowering species that were not characteristic of the specific soil types but appeared in >20% of sampled plots in a region were also included, which added one and three species to the species inventories of Andalusia and California, respectively.

At the onset of the study, we recorded the identity and number of individuals of each animal-pollinated plant species within each plot based on seedling and adult plant identification. Subsequently, the identity of flowering species per plot was revised multiple times along the season. Final values for incidence of flowering species on every plot were obtained by cross-checking the initial list and those recorded during subsequent monitoring. Species with uncertain taxonomy and low frequency of appearance (<10% total regional samples) were not included.

To evaluate how plant species static diversity scales up to affect interactions with diurnal pollinators, within each plot we recorded the total number of open and visited flowers per plant species on three different occasions separated by approximately 14 days along the flowering season (early, mid and late season; see Appendix A in Supplementary material for further details). This sampling scheme was aimed at achieving an estimation of the interaction-effective diversity throughout the entire flowering season at each site, as well as reducing potential biases due to temporal variation in the abundances of particular plant or pollinator species in the community (Sørensen et al., 2011). Notwithstanding, analyzed estimates do not include crepuscular and nocturnal pollinators (Hahn and Brühl, 2016) and reflect a single flowering season which could be affected by the specific abiotic conditions that influenced flowering phenology (e.g., precipitation, temperature) during our study period (but see Appendix A in Supplementary material for a comparison between two consecutive years in Andalusian sites). We recorded visited flowers rather than individual pollinators entering the plot as the former is more relevant for understanding plant pollination success (see Herrera, 2005). The total numbers of visited flowers (i.e. all pollinators combined) and available flowers during the sampling season at each region were: 1318 and 131959 in Andalusia, 4210 and 80965 in California, and 1367 and 20472 in Yucatan.

2.3. Data analyses

We used rarefaction methods to compare diversity among regions and sites within regions because of unequal sampling effort among sites and regions (see Appendices A and B for details). Rarefaction techniques are well known to reduce bias in comparisons that arise from unequal sample sizes among data sets

(Gotelli and Colwell, 2001) and have been used to characterize diversity in plant communities (Cardelús et al., 2006; Collins and Simberloff, 2009), levels of pollinator specialization (Herrera, 2005), and sampling completeness in a plant-pollinator network (Chacoff et al., 2012). Sample-based rarefaction curves (species accumulation obtained by averaging the repeated re-sampling of all pooled samples represented as a function of occurrence, with 95% confidence intervals) were computed using EstimateS 8.0 (Colwell, 2006) with 500 randomizations, sampling with replacement, and 10 samples as the upper abundance limit for rare or infrequent species. In order to evaluate the accuracy of our sampling scheme, we compared the results of the sample-based rarefaction curves with the non-parametric richness estimators which estimate total species richness, including species not present in any sample, and reduce the bias that undersampling imposes on estimated total species richness (Colwell, 2006).

2.3.1. Estimating static plant species diversity

We applied sample-based rarefaction, using species incidence per plot rather than species abundances to avoid the bias introduced by clumped distribution of individuals of some species when sample sizes are small (Collins and Simberloff, 2009). Site-specific and overall (i.e. regional) species richness were estimated by accumulation of plots in a site and in a region, respectively. We compared the results of the sample-based rarefaction curves with the non-parametric richness estimators for incidence data ICE and Chao2 (Colwell, 2006).

2.3.2. Estimating the dynamic floral diversity

To describe the seasonality in diversity of flower availability for each site, we applied rarefaction- and abundance-based non-parametric estimators ACE and Chao1 (Colwell, 2006) to the numbers of open flowers early, in the middle and late in the flowering season.

2.3.3. Estimating the interaction-effective diversity

Sample-based abundance rarefaction curves were obtained from visitation data (i.e. number of visited flowers) to compare plant-pollinator interaction diversity among dates, regions, and sites. We used abundance-based non-parametric estimators of diversity ACE and Chao1 and species accumulation curves were calculated with a more recent method suitable for interpolation and extrapolation that facilitates comparison among datasets that differ substantially in sample size (Colwell et al., 2012). Robustness of estimates among study seasons was examined for the Andalusian sites in the following year with improved monitoring (see Appendix A in Supplementary material for details).
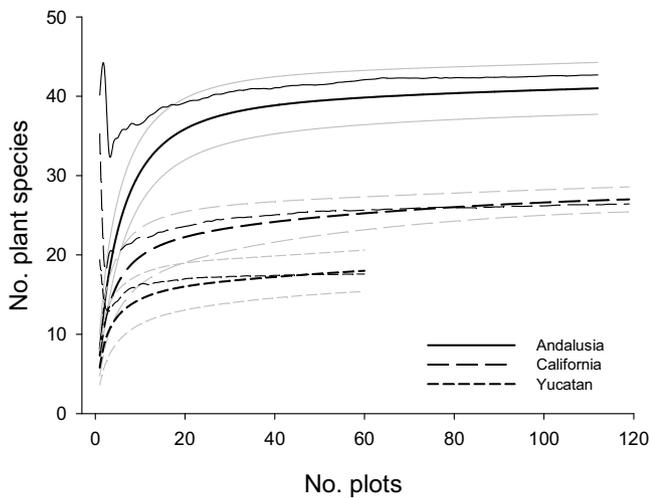
Fig. 1. Rarefaction accumulated plant species curves of the three study flowering communities, sandy limestone-dolomitic outcrops in Andalusia (solid), serpentine seeps in California (long dashed), and sub-tropical coastal scrubland in the Yucatan Peninsula (short dashed), based on presence/absence of species within standardized plots with a sampling design replicated at two levels with 3–5 sites per study region and 20–30 plots per site. Thicker lines designate the observed (Mao Tau) number of species accumulated, grey lines their estimated 95% confidence interval based on 500 randomizations with replacement, and thinner lines the richness rarefaction Chao 2 estimator.

3. Results

3.1. The static view of plant diversity at regional and local scales

Sampling effort was sufficient to characterize static plant species diversity in the three regions: rarefaction estimators were always within the confidence interval of rarefaction accumulated species curves (Fig. 1). The flowering communities associated with sandy dolomite outcrops in Andalusia and dry coastal scrublands in Yucatan were, respectively, the most and least diverse of the three communities studied (Fig. 1; see Appendix C in Supplementary material for the complete list of species).

Estimates of plant species richness per site also stabilized with the sampling effort accomplished, except in the CAP site of Yucatan whose estimate did not reach an asymptote (Appendix B in Supplementary material). Local estimates varied between 11 and 23 species per site and differences among sites within region were higher in Andalusia and California than in Yucatan (Table 1). Overall, the most diverse sites, with ≥ 17 plant species each, included three and four sites from Andalusia and California, respectively (Table 1). The least diverse sites, with ≤14 sp, included all three sites in the Yucatan region with the least rich from the other two regions, altogether suggesting that local (site-level) plant species richness was similar among the three study regions (Table 1). Interestingly, after controlling for sampling effort, cumulative plant species richness within sites was similar to the regional diversity in most cases in California, at one site in Yucatan, but never in Andalusia where regional species diversity was always much higher than at any single site (Appendix B in Supplementary material).

3.2. The dynamic view of floral diversity

Estimates of plant species richness obtained from the accumulated curves of open flowers recorded at three times during the season were coincident to estimates based on species incidence per plot at regional and local scales (Table 1 and Fig. 2 empty bars). When we analyzed seasonality of species with open flowers we found that diversity varied within sites, and asymptotic estimates
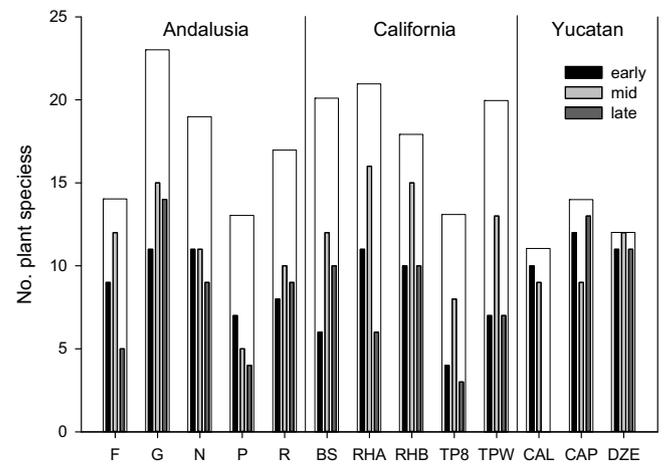

Fig. 2. Seasonal variations in estimates of co-flowering plant species richness at all 13 study sites. Wide empty bars denote the accumulated total species richness per site. Site codes as in Table 1.
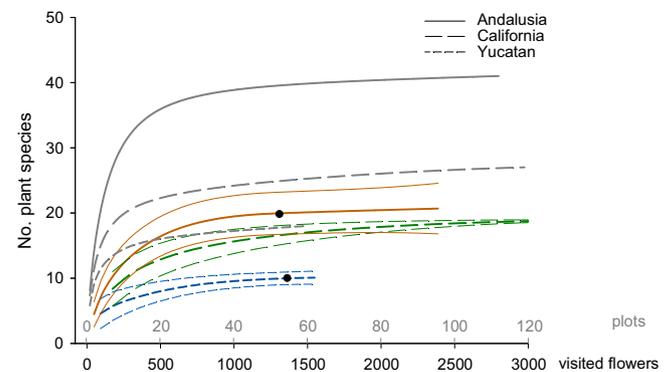

Fig. 3. Static and interaction-effective views of plants diversity. Species accumulation curves of the three study communities, sandy dolomitic outcrops in Andalusia (solid), serpentine seeps in California (long dashed), and sub-tropical coastal scrubland in the Yucatán Peninsula (short dashed), obtained by rarefaction on presence/absence of species (grey) and also by rarefaction and extrapolation of visited flowers (colour) within standardized plots. For visited flowers, both the means and their 95% confidence interval, based on 500 randomizations with replacement, were plotted. Filled dots indicated the actual observations recorded in the least intensively sampled regions, Andalusia (red) and Yucatan (blue). California (green) was the most intensively sampled region (>4000 visits) and extrapolation was thus not required.

of richness of co-flowering species at a given site and period ranged from 3 to 16 (Fig. 2 filled bars). The Yucatan region was unique among the three regions in that species diversity at a given site and date was roughly similar to the total diversity for that site through the entire study period (Fig. 2). Patterns of seasonal abundance were more variable in Andalusia and California. For example, some sites exhibited marked seasonal changes in numbers of co-flowering species among dates that peaked at mid-season (e.g., seeps BS, RHA, TP8 and TPW in California), while others displayed similar diversity among dates (e.g., G, N and R outcrops in Andalusia) but never reached the global diversity at each particular site (Fig. 2).

3.3. The interaction-effective view of diversity

Rarefaction analyses indicated that despite substantial variation in the number of observed visits among regions the number of plant species accumulated from visitation records still stabilized with the achieved sampling effort (Fig. 3). The diversity estimated from visited flowers was consistently lower than the corresponding esti-

mates obtained from species incidence (*static*) and open flowers (*dynamic*) and, in all three communities the interaction-effective accumulated species never reached that obtained from incidence of species (Fig. 3). Even in California, the best sampled community after >252 h observation and >4000 visits recorded, we did not observe visitation for 8 out of 27 flowering plant species, an estimate that asymptotically stabilized at much lower sample size than actual sample size. Even more interesting perhaps is that the interaction-effective diversity reflected much more impoverished communities in Yucatan, where only about half the species were visited despite >1300 recorded visits in 184 h of observation (Fig. 3). Finally, the interaction-effective diversity in Andalusia, despite the lowest monitoring effort and moderate records obtained (1318 visits in 32.4 h observation), stabilized at the actual sample size and remained the highest among the three communities, although without differing from California estimates (Fig. 3). Most remarkably the asymptotic estimate of species diversity obtained from visitation was roughly half the static diversity of the community and extrapolation indicated that a moderate increase in sampling effort in the study year would not greatly alter the results (Fig. 3). Finally, a largely improved observation scheme in the following year (>10,000 visits) indicated that a very intensive monitoring can improve the estimate of interaction-effective diversity, which may vary among seasons, and corroborated that interaction-effective diversity remained lower than the static one (see Appendix A in Supplementary material for details).

4. Discussion

In this paper we propose that by analyzing not only diversity of plants flowering across the season, but also diversity of floral resources available and actually used by pollinators at narrower spatial and temporal scales we can effectively explore pollination complexity at the community level. Our rarefaction analyses of diversity of entomophilous plant communities at three geographically distant diversity hotspots revealed important differences in diversity analyzed from static, dynamic and pollinator mediated perspectives.

4.1. How does our perspective of diversity affect our comparisons among communities?

The traditional—species inventory-based—static estimates of plant diversity, indicated significant differences among the three communities in diversity of plants flowering across the season that increased with landscape heterogeneity. The dynamic and interaction-effective views of diversity which interpreted this maximum taxonomic diversity from the plant-pollinator interaction perspective indicated much smaller differences among them associated to seasonal partitioning in the richest communities and a largely incomplete use of available resources by diurnal pollinators at all three regions.

In particular, the two Mediterranean, soil-specific flowering communities exhibited higher static species diversity at regional and local scales than the dry subtropical scrubland community. The globally richest community associated to sandy dolomite soils in Andalusia was characterized by wide variation in local species diversity and a stronger difference between average local diversity and overall regional accumulated species richness which suggests increased dissimilarity in species composition among study sites (C. Alonso unpubl. results). Such spatial heterogeneity in community composition is characteristic of the highlands flora of the Baetic ranges, most likely due to heterogeneity of soil properties combined with altitudinal variance and reduced connectivity in this mountainous landscape (Mota et al., 2002, 2008). A large number

of studies on Californian serpentine soils have provided a clear-cut classification of associated soil-based distinctive plant communities (Freestone and Inouye, 2006; Alexander et al., 2007) which is still missed for this Andalusian flora. Thus, a more thorough understanding of local ecological filters (soil, radiation, canopy cover, geographic isolation) within Andalusia dolomite outcrops would help to conclude if the stronger spatial structure of plant diversity here documented is characteristic of this mountainous environment or the apparent differences with Californian serpentines have resulted from unequal classification accuracy of the two habitat-specialist communities. Reduced spatial heterogeneity was characteristic of the dry subtropical coastal scrublands studied at Yucatan Peninsula, a narrow bioclimatic well-defined area where human disturbances are common (Espadas Manrique et al., 2003), which happens to be the least diverse across study regions. There, the three study sites were similar in species richness and composition, with local (site-level) species richness reaching similar values than the least diverse sites in Andalusia and California.

Interestingly, subtropical coastal scrublands were also the least variable in species number and composition along the season, likely because in these tropical dry forests phenological control by water stress is prevalent and the fast response to seasonal rainfall promotes synchrony within populations and among species (Bullock, 1995). Conversely, in the Mediterranean it is usually winter precipitation that has a delayed effect on the onset of the spring flowering season, and the predictable change from very moist soils early in the season to very dry soils by late summer would contribute to seasonal changes in species flowering, as some species are more or less tolerant to seasonal drying (Alexander et al., 2007). Thus, seasonality resulted in decreased diversity of local co-flowering communities at the two Mediterranean ecosystems, that eventually include just 3–6 species and only at particular dates along the season peak to >10 species, the maximum obtained at the subtropical community (Fig. 2). Such values are even lower than reported for daily flowering data in some European grasslands (Fründ et al., 2010), suggesting that, in contrast to commonly presumed, daily co-flowering communities are not necessarily more diverse at biodiversity hotspots than at other regions. A stronger seasonal niche partitioning in the richest communities could actually promote plant species coexistence (Benadi et al., 2012) and modulate the relationship between static and interaction-effective diversities.

4.2. Could local plant diversity be functionally limited from the pollinators' perspective?

Our rarefaction analyses of visited flowers revealed a considerable 'diversity shrinkage' of all three plant communities studied when diversity was assessed from the perspective of pollinators use. Thus, differences among communities in interaction-effective diversity were lower than observed with the traditional static view. Optimization of diurnal pollinator observation effort can increase the sampling success (Hegland et al., 2010; Alonso et al., unpubl. data and Appendix A in Supplementary material) and, eventually, the study of nocturnal pollinators may improve the assessment of this interaction-effective diversity (Hahn and Brühl, 2016). Nevertheless, even when visitation records are as high as those observed in California, the effective diversity of flowering communities visited by pollinators was significantly lower than available (see also Chacoff et al., 2012). Results were even more extreme in the other two regions where pollinator visitation records were lower although sufficiently high to characterize the community. In the study year, pollinators in Andalusia and Yucatan visited only half of the overall number of species flowering. Reduced interaction-effective diversity observed in three widely distant regions suggested that despite the intrinsic variability of plant-pollinator interactions among study seasons (Lázaro et al.,

2010), the diversity of plants functionally related by interactions with pollinators must be usually lower than available, but this pattern will only be acknowledged if unvisited species are recorded (see also Ebeling et al., 2008).

Determining the spatial and temporal scale at which the discordance is largest between static and interaction-effective diversity may help clarify outstanding issues in plant pollinator networks, such as why pollinator richness is larger than plant richness (Vázquez et al., 2009) and how seasonal mismatch between flowers and pollinators contributes to artifactual nestedness (Burkle and Alarcón, 2011; Olesen et al., 2011). Our findings suggest that disparity between floral resources available and those actually used not only arises in highly heterogeneous plant communities as exemplified by dolomite outcrops in Andalusia, but also in the least spatially and seasonally variable subtropical plant community, and thus could also depend on the details of identity, diversity and behavior of pollinators (see e.g. Fontaine et al., 2006; Burkle et al., 2016) which in turn may also change among years within a certain location (Appendix A in Supplementary material). Altogether, diversity of plant-pollinator interactions might be relatively constrained at local scales and become more variable in the identity of the species involved in highly diverse regions (see e.g., Petanidou et al., 2008). The corollary may follow that diversity *per se* holds as an added value of a given community, worth preserving not only from ethical or practical issues – the hotspot conservation approach – but also because it substantiates functionality of interactions at broader spatial and temporal scales than they actually occur (Balvanera et al., 2006; Blüthgen and Klein, 2011). The effect that flowers of unvisited species never included in pollination-networks may have on pollinators' activity at different spatial and temporal scales remains largely unknown but increased numbers of flowering species in a plot tend to increase frequency of visits and further decrease variance on records at different observation periods suggesting they could be important for stability of pollination service (Ebeling et al., 2008).

In conclusion, understanding plant-pollinator interactions in highly diverse communities requires an intensive sampling effort but relevance of predicted indirect competition or facilitation associated to diversity at global scales (Vamosi et al., 2006; Alonso et al., 2010) and their consequences for ecosystem services (Díaz and Cabido, 2001) makes the challenge worthwhile. With a simplified rarefaction method we found that the effective diversity of visited flowers in three geographically distant diversity hotspots differing in maximum taxonomic diversity and structure was consistently low at the narrow spatial and temporal scales suitable for indirect interactions. A rarefaction assessment of effective interactions at different scales, as the one illustrated here, may help to identify the most critical sources of variation from the perspective of multitrophic interactions at different communities.

Author Contributions

CA, VPT and TLA conceived the ideas; all authors collected the data and made preliminary analyses; CA and GAG analyzed the data; CA led the writing and all authors contributed to refinement.

Acknowledgments

Appendix A[{(Appendices A–C)}] Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.flora.2016.10.001.

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