

BRIEF COMMUNICATION

Variation in sampling effort affects the observed richness of plant–plant interactions via heterospecific pollen transfer: implications for interpretation of pollen transfer networks

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PREMISE OF THE STUDY: There is growing interest in understanding plant–plant interactions via pollen transfer at the community level. Studies on the structure and spatial variability of pollen transfer networks have been valuable to this understanding. However, there is high variability in the intensity of sampling used to characterize pollen transfer interactions, which could influence network structure. To date, there is no knowledge of how sampling effort influences the richness of pollen on stigmas and thereby transfer interactions observed, nor how this may vary across species and study sites.

METHODS: We use rarefaction curves on 16 species to characterize the relationship between sampling effort (number of stigmas analyzed) and the richness of pollen transfer interactions recorded. We further assess variability in this relationship among species, plant community types, and sites within a single plant community.

KEY RESULTS: We show high among-species variation in the amount of sampling required to sufficiently characterize interspecific pollen transfer. We further reveal variability in the sampling effort–interaction richness relationship among different plant communities and even for the same species growing in different sites.

CONCLUSIONS: The wide heterogeneity in the sampling effort required to accurately characterize pollen transfer interactions observed has the potential to influence the characterization of pollen transfer dynamics. Thus, sampling completeness should be considered in future studies to avoid overestimation of modularity and specialization in pollen transfer networks that may bias the predicted causes and expected consequences of such processes for plant–plant interactions.

KEY WORDS dolomite outcrops; heterospecific pollen; interaction networks; pollen transfer; pollination; pollinator sharing; rarefaction curves; serpentine seeps; Yucatán peninsula.

Findings of generalization in plant–floral visitor networks has led to a renewed interest in the consequences of high levels of pollinator sharing among plant species. One particular consequence of pollinator sharing is the frequent transfer of pollen between different plant species, i.e., heterospecific pollen transfer (hereafter HP transfer; Morales and Traveset, 2008). It has been well documented that

HP transfer can have substantial fitness effects (reviewed in Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013; Arceo-Gómez and Ashman, 2016) with potential evolutionary consequences (Ashman and Arceo-Gómez, 2013; Arceo-Gómez et al., 2016b). Moreover, recent studies have focused on unraveling patterns of multiple-species interactions in a community via HP transfer and

determining the factors mediating the structure of such interactions (e.g., Fang and Huang, 2013; Emer et al., 2015; Tur et al., 2016; A. L. Johnson and T.-L. Ashman, unpublished data), which is important to understand the landscape of these interactions in nature. To do this, studies have relied on network analysis tools that have been used to describe other community-level interspecific interactions such as plant–frugivore (e.g., Jordano et al., 2003), host–parasite (e.g., Vázquez et al., 2005), plant–mycorrhiza (e.g., Montesinos-Navarro et al., 2012), plant–herbivore (e.g., López-Carretero et al., 2014) and plant–floral visitor interactions (e.g., Olesen and Jordano, 2002). Heterospecific pollen transfer networks, in particular, have been shown to exhibit a modular structure (i.e., groups of species that interact more strongly with each other than with species in other groups; Olesen et al., 2007) and be more specialized than the more commonly characterized plant–pollinator visitation networks (e.g., Olesen and Jordano, 2002; Vázquez and Aizen, 2004; Fang and Huang, 2013; Emer et al., 2015). Within HP networks, a small subset of species that act as ‘hub-pollen receptors’ exists (species that receive HP from many species), and can be a different subset from those that act as ‘hub-pollen donors’ (species that donate pollen to many species; e.g., Fang and Huang, 2013; Tur et al., 2016). This suggests that most species are ‘specialized’ with respect to whether they receive or donate HP and that these interactions tend to be asymmetrical (Fang and Huang, 2013). Understanding HP network structure is important because it can give important insights into true levels of pollinator generalization and specialization in plant communities, as well as into the potential for competitive and facilitative plant–plant interactions via pollinators (e.g., Tur et al., 2016). Furthermore, understanding patterns of HP transfer at the community level can shed light into the ecological and evolutionary consequences of plant–plant interactions because HP has the potential to play an important role in driving floral character displacement and in mediating plant community assembly (Ashman and Arceo-Gómez, 2013). Overall, HP networks have proven to be valuable in advancing our understanding the mechanisms that mediate the assembly of co-flowering communities and how these may vary spatially (Emer et al., 2015; Tur et al., 2016).

HP networks, however, as with other types of species interaction networks, are subject to sampling bias and statistical limitations associated with insufficient sampling effort (Vázquez et al., 2009; Blüthgen, 2010; Rivera-Hutinel et al., 2012; Kuppler et al., 2017). Surprisingly, the number of stigmas observed to score HP deposition has been highly variable both within and across studies of HP transfer networks (3 to >30 stigmas per species; e.g., Fang and Huang, 2013; Emer et al., 2015; Tur et al., 2016), and in some cases the sampling effort for the species studied is not clearly disclosed. For instance, the frequency and diversity of HP interactions for a single plant species in a network has been determined from as little as three (Emer et al., 2015), to 15 (Fang and Huang, 2013) and possibly more than 25 stigmas (Tur et al., 2016). In some cases, these stigmas have been collected from only one (Emer et al., 2015), five or 15 individual plants (Fang and Huang, 2013). This reduced and unequal sampling is worrisome given that a key assumption in network analysis is that the observed interactions constitute an adequate sample of the entire set of interactions present in the community. Furthermore, a large body of literature has documented how sampling effort can have a strong influence on the probability of observing species interactions (e.g., Herrera, 2005), thus affecting the total number of links in the overall network structure (e.g., Goldwasser and Roughgarden, 1997; Nielsen and Bascompte, 2007; Vázquez

et al., 2009; Rivera-Hutinel et al., 2012; Kuppler et al., 2017). Thus, limited sampling could lead to the mischaracterization of network structure, and thus misinterpretation of patterns of specialization and modularity (e.g., Kuppler et al., 2017). This is particularly important in HP transfer networks because intraspecific variation in HP receipt can be large (Arceo-Gómez et al., 2016a). For instance, variation in HP load size and diversity among individuals within a single species accounted for up to 50% of the total variation in HP receipt observed across 19 species in three different plant communities (Arceo-Gómez et al., 2016a). Such high intraspecific variability can contribute to biases due to small sampling effort, for instance if by chance the few stigmas observed had no or little HP on them. In addition, studies have revealed considerable variation in the richness of HP transfer interactions observed for a single species growing in different sites (e.g., Arceo-Gómez and Ashman, 2013). This among-site variation could suggest that the number of stigmas required to characterize pollen transfer interactions for a single species may be different depending on the study site. To our knowledge, however, this has not been considered even as studies start to evaluate spatial variability in pollen transfer network structure (e.g., Emer et al., 2015; Tur et al., 2016). Overall, there is no knowledge of how sampling effort (number of stigmas observed) influences the probability of detecting interactions via HP transfer, and how this varies among different plant species, sites, and plant community types. As a result, there is no comparative baseline for determining the minimal amount of sampling required to satisfactorily characterize HP transfer networks.

Rarefaction curves have been typically used to quantify the degree of sampling completeness in a network (e.g., Chacoff et al., 2012; Rivera-Hutinel et al., 2012; Koski et al., 2015) and thus avoid, or at least acknowledge (e.g., Chacoff et al., 2012), potential sampling bias on the characterization of network structure. Rarefaction techniques are useful to estimate potential bias that arises from unequal sample sizes (Gotelli and Colwell, 2001). This analytical technique has been used widely to characterize sampling completeness in studies evaluating patterns of diversity in plant communities (e.g., Cardelús et al., 2006; Collins and Simberloff, 2009; Alonso et al., 2016), levels of pollinator specialization (Herrera, 2005), and for assessing sampling completeness in plant–floral visitor networks (e.g., Chacoff et al., 2012; Koski et al., 2015; Parra-Tabla et al., 2017b). Thus, in this study we use rarefaction analysis to evaluate the relationship between the number of stigmas sampled and the richness of plant–plant interactions on the stigma observed in 16 species—each individual species located in one out of three distinct geographic regions (i.e., California, Andalusia, Mexico; see below). We use rarefaction curves to assess how the sampling effort–HP richness relationship varies among plant species and plant community types (geographic regions). We further assess among-site variation in the sampling effort–HP richness relationship for the same species within the same geographic region. Finally, we discuss the implications of our findings for the characterization of HP transfer networks, and our understanding of plant–plant interactions mediated by pollen transfer.

MATERIALS AND METHODS

Study system

The relationship between HP receipt and sampling effort was evaluated in 16 species sampled in three geographically and ecologically

distinct co-flowering plant communities: sandy limestone-dolomitic outcrops in Andalusia, Spain (DO; 37°56'N/02°50'W), dry scrublands in Yucatán, Mexico (DS; 21°17'N/89°35'W) and serpentine seeps in California, USA (SS; 38°51'N/122°25'W) (Table 1; also see Alonso et al. 2016). In Andalusia, the study took place at the Cazorla Natural Park, in the Yucatán along the north coast of the peninsula and in California at the McLaughlin Natural Reserve. All three communities have seasonal flowering seasons and are species-rich communities (38–67 species; Arceo-Gómez et al., 2016a), dominated by small herbaceous perennials and annuals (Estrada-Loera, 1991; Medail and Quezel, 1999; Freestone and Inouye, 2006; Alonso et al., 2013; Parra-Tabla et al., 2017a). The study was conducted during the main flowering season, which lasts for 2–3 months at all the studied communities (June–July in SS, May–June in DO, July–Sep in DS). Because flowering at all these communities is highly dependent on water availability (e.g., seasonal rainfall), there is a high degree of flowering overlap among species within each community

(Alonso et al., 2016; Arceo-Gómez et al., 2016a). For a full description of these plant communities see Alonso et al. (2016). Four, five, and seven insect-pollinated plant species representing nine plant families were sampled in DS, SS, and DO plant communities respectively (Table 1).

Sample collection

For this study, we analyzed the sampling effort–HP richness relationship for a subset of the species and stigmas observed in Arceo-Gómez et al. (2016a). In this study, we only analyzed stigmas of species within each of the three plant community types (DO, DS, SS) that were collected from the same site ('RHA', 'G', and 'Capilla' in Alonso et al., 2016). Thus, all stigmas within each community type were exposed to the same pollination environment. In total we analyzed pollen data from between 11–164 stigmas per species (1081 total; Table 1) collected from individuals spatially spread along linear

TABLE 1. Data for 16 plant species studied across three plant communities: dolomite outcrops (DO) in Spain, dry scrublands (DS) in the Yucatán, and serpentine seeps (SS) in California. Data is presented on sample size (number of stigmas sampled), average number of heterospecific pollen (HP) grains per stigma, total number of HP morphotypes, 95% confidence intervals (CI) for the rarefaction curves, and the nonparametric species richness estimator (Chao 1). Information on plant family and the code (see Fig. 1) for each species is also given.

Community	Species	Family	Species code	Sample size	Average number of HP grains	Total number of HP types	Rarefaction 95% lower CI	Rarefaction 95% upper CI	Chao 1 estimator
DO	<i>Fumana baetica</i> (Güemes)	Cistaceae	FB	56	5.3	4	3.06	4.94	4
DO	<i>Fumana paradoxa</i> (Heywood)	Cistaceae	FP	64	9.4	11	6.28	15.72	11
DO	<i>Helianthemum cinereum</i> (Pers.)	Cistaceae	HCI	131	2	9	7.46	10.54	9
DO	<i>Helianthemum appeninum</i> (L.) Mill.	Cistaceae	HCR	47	1.04	4	2.68	7.32	5
DO	<i>Silene psammitis lasiostyla</i> (Link)	Caryophyllaceae	SPL	11	35.2	13	8.77	17.23	15.9
DO	<i>Thymus orospedanus</i> (Villar)	Lamiaceae	TO	149	0.4	7	3.22	10.78	7.2
DO	<i>Teucrium polium</i> (L.)	Lamiaceae	TP	164	0.8	13	8.98	17.02	22.9
DS	<i>Cuphea gaumeri</i> (Koehe)	Lythraceae	CG	39	0.69	2	2	2	2
DS	<i>Cienfuegosia Yucatánensis</i> (Millsp.)	Malvaceae	CY	100	4.07	8	8	8	8
DS	<i>Sida acuta</i> (Burm.f.)	Malvaceae	SA	75	0.53	3	1.31	4.69	3
DS	<i>Angelonia angustifolia</i> (Benth)	Plantaginaceae	AN	61	3.8	2	2	2	2
SS	<i>Delphinium uliginosum</i> (Curran)	Ranunculaceae	DU	48	206.5	9	7.16	10.84	9
SS	<i>Mimulus guttatus</i> (Fisch.)	Phrymaceae	MG	33	25.2	14	10.58	17.42	19.9
SS	<i>Mimulus nudatus</i> (Curran ex Greene)	Phrymaceae	MN	47	12.1	11	9.14	12.86	11
SS	<i>Triteleia peduncularis</i> (Lindl.)	Liliaceae	TR	28	123.9	12	10.67	13.33	12
SS	<i>Zigadenus venenosus</i> (S.Watson)	Liliaceae	ZV	28	8.1	8	4.71	11.29	12.4

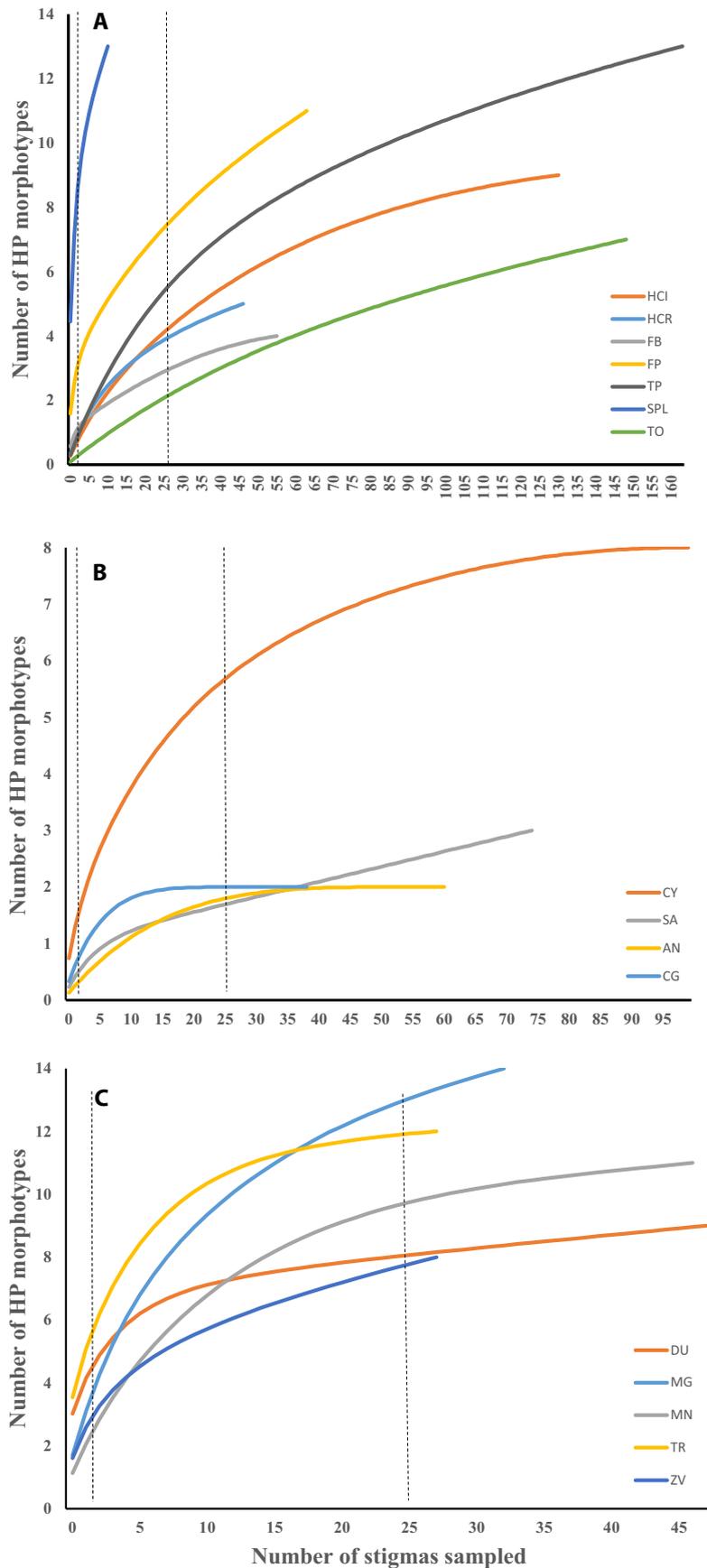


FIGURE 1. Sample-based rarefaction curves of plant–plant interactions via heterospecific pollen (HP) deposited on stigmas of 16 plant species in three plant communities: (A) dolomite outcrops in Spain, (B) dry scrublands in Mexico, and (C) serpentine seeps in California, USA. For clarity, confidence intervals are not included in the graphs, but all confidence intervals are given in Table 1. The approximate minimum and maximum (suggested) number of stigmas that have been sampled for characterizing HP transfer networks (3–25 stigmas) are shown (vertical dotted lines). For full plant species names and their respective codes see Table 1.

transects, with just 1–2 stigmas analyzed per individual. To evaluate among-site variation within the same community type (i.e., geographic region) we scored HP grains in 11, 87, and 110 *Silene psammittis lasiostyla* stigmas in three different sites within the dolomitic outcrop community in Spain (Table 2; Alonso et al., 2016). In this species, each stigma was collected from a different individual. For more information on stigma collection and processing, see Arceo-Gómez et al. (2016a). The number and identity of each pollen grain on the stigma was recorded with the aid of a light microscope (Carl Zeiss, Germany). A pollen library was constructed for each community to aid in the identification of pollen grains on stigmas to the species or morphotype level (Arceo-Gómez et al., 2016a). A total of 16, 20, and 32 pollen morphotypes were identified in DS, SS, and DO plant communities, respectively (Arceo-Gómez et al., 2016a).

Data analyses

We used rarefaction methods to evaluate the relationship between the number of stigmas sampled and the total number of unique plant–plant interactions via HP transfer (number of HP morphotypes) observed for each species. Each stigma was considered a sampling unit and the total number of stigmas as the total sampling effort. Sample-based rarefaction curves for abundance data and 95% confidence intervals were computed for each species using EstimateS 9.1 (Colwell, 2006) with 500 randomizations and sampling without replacement. In order to evaluate the sufficiency of our sampling, we compared the expected richness of HP transfer interactions to the observed number of interactions using a nonparametric richness estimator commonly used for abundance data (Chao 1; Colwell, 2006; Chao et al., 2009). Confidence intervals (CI) for the rarefaction curves are not shown in the figures for clarity, but all CI values are shown in Tables 1 and 2.

RESULTS

We observed wide variation in average HP per stigma (0.4–206.5 HP grains) and in the total number of HP morphotypes observed on stigmas (2–14) across all the plant species studied (Table 1; Fig. 1). Rarefaction analyses further revealed large among-species variation in the

TABLE 2. Sample size (number of stigmas sampled), average number of heterospecific pollen (HP) grains per stigma, total number of HP morphotypes, 95% confidence intervals (CI) for the rarefaction curves, and the nonparametric species richness estimator (Chao 1) for *Silene psammitis lasiostyla* stigmas collected at three different sites within the dolomitic-outcrop plant community in Andalusia, Spain. Each site is given a code in Figure 2.

Site name	GPS coordinates	Site code	Sample size	Total number of HP types	Rarefaction 95% lower CI	Rarefaction 95% upper CI	Chao 1 estimator
Arenales del Guadalentín	37°55'N/02°50'W	G	11	13	8.77	17.23	15.9
Fuente Bermejo	37°56'N/02°50'W	F	87	22	15.14	28.86	31.9
Nava de las Correhuelas	37°56'N/02°52'W	N	110	24	20.81	27.19	25

number of samples (stigmas) needed to fully capture the richness of HP transfer interactions on each study site. For instance, in the dolomite outcrops in Andalusia, we captured the full range of HP transfer interactions in *Fumana baetica* (i.e., no difference in the observed and expected number of interactions; Table 1) after sampling 56 stigmas, but we only recorded 56% of all possible interactions in *Teucrium polium* after observing more than 160 stigmas at this same site (Table 1; Fig. 1). In the serpentine seeps in California, sampling 28 stigmas resulted in 100% of all possible HP transfer interactions recorded for *Triteleia peduncularis*, but only 64% for *Zigadenus venenosus* (Table 1). Moreover, the rate at which the sampling effort-HP richness relationship saturates varied broadly among study species, although in most cases saturation arrives with sample sizes well above the ranges most frequently used (Fig. 1). For instance, in Andalusia, the number of HP transfer interactions starts to saturate after observing 40 stigmas for *Fumana baetica* (FB), but it takes more than 100 stigmas for *Thymus orospedanus* (TO), *Helianthemum cinereum* (HCI), and *Teucrium polium* (TP; Fig. 1A). On the Yucatán Peninsula, the number of HP transfer interactions saturates for *Cuphea gaumeri* (CG) and *Angelonia angustifolia* (AN) after sampling 25 stigmas or less, but it takes more than 50 stigmas for the remaining two species (Fig. 1B). In California, the sampling effort-HP richness relationship for *Triteleia peduncularis* (TR), *Mimulus nudatus* (MN), and *Delphinium uliginosum* (DU) starts saturating after sampling 25 stigmas, however we did not seem to reach saturation for *Mimulus guttatus* (MG) and *Zigadenus venenosus* (ZV) after sampling the same number of stigmas (Fig. 1C). The nonparametric (Chao 1) species richness estimator further confirmed the incompleteness of our sampling for some species (Table 1). For instance, we only captured 56%, 64%, and 70% of all expected interactions (Chao 1) via HP transfer for TP, ZV, and MG after sampling 164, 28, and 33 stigmas, respectively (Table 1; Fig. 1). Interestingly, in the dry scrubland community in Yucatán, we captured 100% of all interactions for all species even though sampling effort varied almost three-fold among species (Table 1).

We also observed high among-site variation in the sampling effort needed to fully capture the richness of HP transfer interactions for a single species. In particular, we captured 81% of all possible HP transfer interactions for *Silene psammitis lasiostyla* at 'Arenales del Guadalentín' (site G in Fig. 2) with only 11 stigmas. However, we only recorded 68% of all interactions for this same species at 'Fuente Bermejo' (site F in Fig. 2) even after sampling more than 80 stigmas (Table 2). At 'Nava de las Correhuelas' (site N in Fig. 2) we captured 96% of all interactions with a sample of 110 stigmas (Table 2). Consequently, we also observed among-site variation in the rate at which the sampling effort-HP richness relationship saturates. Specifically, it took approximately 30 *S. psammitis lasiostyla* stigmas to reach a saturation point at 'Nava de las Correhuelas' (site N in Fig. 2), but we did not reach a saturation point at 'Fuente Bermejo' (site F in Fig. 2).

DISCUSSION

Sampling effort (number of stigmas) strongly affected the probability of accurately characterizing the richness of plant-plant interactions via HP transfer (Fig. 1). Specifically, large among-species variation in the degree to which sampling can affect the richness of HP transfer interactions was observed within each plant community studied (Table 1, Fig. 1). The high among-species differences observed in the amount of sampling needed to capture the full range of HP interactions could be the result of differences in the efficiency of the pollinator community, i.e., where some species receive constant loads of HP receipt with every pollinator visit while others receive unpredictable HP loads across flowers (e.g., Arceo-Gómez et al., 2016a). As an alternative, it may also result from variation in floral traits that can influence patterns of HP deposition (e.g., display size, stigma size, and/or flower symmetry; McLernon et al., 1996; Montgomery and Rathcke, 2012; Arceo-Gómez et al., 2016a; see below). Regardless of the underlying mechanism, the heterogeneity in the sampling effort required to fully characterize HP transfer interactions across species has the potential to influence the structure of pollen-transfer networks and suggests that having the same amount of sampling for all species may not guarantee the reliability of diversity estimates of HP transfer interactions (e.g., Herrera, 2005; Dorado et al., 2011). It is apparent that sampling less than 15 stigmas was insufficient to capture the full range of HP interactions across all species and

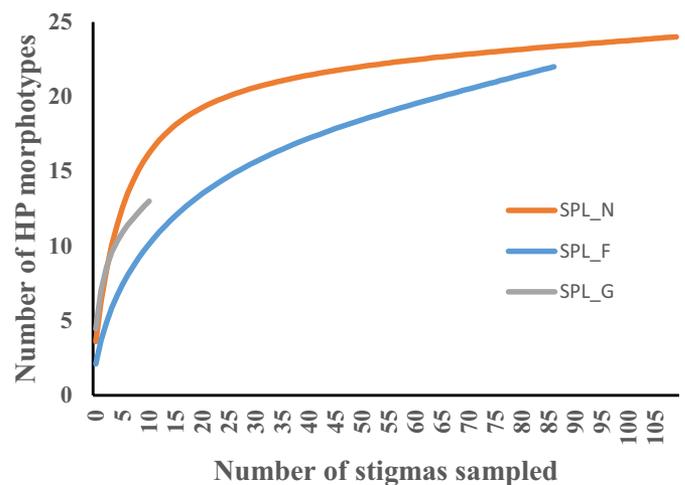


FIGURE 2. Sampled-based rarefaction curves of plant-plant interactions via heterospecific pollen (HP) deposited on stigmas of *Silene psammitis lasiostyla* (SPL) at three different sites within the dolomitic-outcrop plant community in Andalusia, Spain: Arenales del Guadalentín (site G), Fuente Bermejo (site F), and Nava de las Correhuelas (site N). For clarity, confidence intervals are not included in the graph, but all confidence intervals are given in Table 2.

plant communities studied (Fig. 1). This level of undersampling would have resulted in an overall poor estimation of the diversity of interaction partners for any species in any of our study systems, which in turn can lead to the overestimation of modularity and specialization in a network (e.g., Blüthgen et al., 2008; Blüthgen, 2010; Dorado et al., 2011). For instance, Blüthgen et al. (2008) showed via simulation that low sampling leads to overestimation of network specialization because poorly sampled species are always regarded as ‘specialists’. It has also been shown that increasing network connectance by detecting new interactions (for instance with more sampling), can lead to a decrease in network modularity because individual species in a network become more connected (Olesen et al., 2007). Avoiding these biases in the characterization of network structure is essential because new studies are starting to reveal its importance in mediating ecological processes that are relevant for ecosystem function and stability (e.g., Dunne et al., 2002; Memmott et al., 2004; Kaiser-Bunbury et al., 2010; Tylianakis et al., 2010). Thus, based on the lowest amount of sampling required here to fully characterize HP interactions for at least one species (Fig. 1), we suggest sampling a minimum of 25–30 stigmas per plant species (e.g., Tur et al., 2016) when characterizing the richness of HP interactions. We are aware that this level of sampling may not be ideal across all species, however, it establishes a minimum baseline that could help avoid extreme undersampling of plant–plant interactions via HP transfer. Ideally, rarefaction in the focal study system would provide direct evidence of the depth of sampling needed. Identification of the individual species characteristics that drive variation in patterns of HP receipt would also aid in the design of strategies to capture the full range of HP interactions across species. In this sense, the amount of intraspecific variation (i.e., variation among plants of the same species) in the size and diversity of the HP load could determine the amount of sampling required to capture the whole range of HP transfer interactions. Specifically, we can expect that as intraspecific variation in HP receipt (load size and diversity) increases the amount of sampling required to capture all possible interactions would also increase. Interestingly, in the species studied here, the degree of intraspecific variation in HP load size and diversity is not affected by flower size, flower longevity, flower symmetry, flower abundance, or floral visitor diversity (Arceo-Gómez et al., 2016a). This suggests that neither of these floral traits nor pollinator-species richness would be useful for determining which species will require more sampling. For instance, in California the number of HP transfer interactions sampled saturates faster in *M. nudatus* than in *M. guttatus* (Fig. 1) even though their flower morphology is practically identical. Furthermore, in Yucatán, complete characterization of HP transfer interactions required 50% more sampling in *Cienfuegosia yucatanensis* (CY) compared to *Cuphea gaumeri* (CG; Fig. 1B) even though the diversity of the floral visitor community is similar between them (Simpson’s diversity index: 2.1 and 2.4 respectively; Arceo-Gómez et al., 2016a). Instead, our analysis of *S. psammithis lasiostyla* suggests that intraspecific variation in HP receipt may depend on variation in the efficiency of the pollinator community and the rate at which they deposit conspecific versus heterospecific pollen at each site (also see Arceo-Gómez et al., 2016a). However, while variation in mean HP load size and diversity among species within a single community has received some attention (e.g., Montgomery and Rathcke, 2012; Arceo-Gómez et al., 2016a), very few studies have evaluated the magnitude and drivers of intraspecific variation in patterns of HP receipt. Thus, more studies are needed that evaluate drivers of intraspecific variation in patterns of HP receipt, which

would aid in designing efficient strategies for HP sampling across species. Finally, we emphasize the need for studies that evaluate the fitness effects of HP receipt (e.g., Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2016) along with HP transfer dynamics, in order to fully understand the potential consequences of changes in HP network structure.

It also important to point out that the degree to which sampling effort affects the detection of HP transfer interactions could be exacerbated by collecting stigmas from the same individual, because intraspecific variation in HP receipt has been shown to be larger than interspecific variation (Arceo-Gómez et al., 2016a). Furthermore, Kuppler et al. (2017) showed that high intraspecific variation coupled with low sampling effort leads to unreliability of aggregated network statistics such as modularity. On the other hand, sampling at the individual level would also increase the degree of spatial heterogeneity captured within a plant population, potentially capturing more variation in pollinator assemblages and a higher number of HP morphotypes. Thus, we suggest that sampling stigmas across many individuals instead of sampling multiple stigmas within the same individual would perhaps yield the best results when evaluating HP transfer networks.

We also observed variation among plant communities in the degree to which sampling affects the richness of HP transfer interactions (Table 1; Fig. 1). Such differences among communities could be due to intrinsic differences in the density and diversity of plant and pollinator communities at each region and site. For instance, accumulated plant species richness in the DO plant community is twice as large as in the SS community and almost three times higher than in the DS plant community (Alonso et al., 2016). Plant species richness at the individual sites studied here was also higher in DO compared to SS and DS sites (23, 21, and 14 species, respectively; Alonso et al., 2016). Differences in the total number of co-flowering plant species in a community have been shown to underlie variation in patterns of HP receipt (e.g., Arceo-Gómez and Ashman, 2013). Finally, we observed high among-site variation to the degree to which sampling affects the richness of HP transfer interactions for a single species (Table 2; Fig. 2). Thus, our results suggest that the level of generalization in HP transfer interactions may not be a species property (e.g., Herrera, 2005) and the sampling effort needed to accurately characterize HP transfer interactions may not only depend on the species but on the particular site studied. However, to our knowledge, this has never been considered when evaluating spatial variation in HP transfer networks.

CONCLUSIONS

In conclusion, this study highlights the importance of considering the degree of sampling when evaluating the richness of species interactions and suggests that not all species and communities require the same amount of sampling to accurately characterize the diversity of HP transfer interactions. Thus, we emphasize that studies that evaluate interactions that occur via HP transfer need to adhere to the same standard as those evaluating other species interaction networks (e.g., Dorado et al., 2011; Chacoff et al., 2012; Rivera-Hutinel et al., 2012; Koski et al., 2015).

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SUPPORTING INFORMATION

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

LITERATURE CITED

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