

INVITED PAPER

For the Special Issue: Ecology and Evolution of Pollen Performance

# Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization<sup>1</sup>

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**PREMISE OF THE STUDY:** Coflowering plants are at risk for receiving pollen from heterospecifics as well as conspecifics, yet evidence shows wide variation in the degree that heterospecific pollen transfer occurs. Evaluation of patterns and correlates of among- and within-species variation in heterospecific pollen (HP) receipt is key to understanding its importance for floral evolution and species coexistence; however, the rarity of deeply sampled multispecies comparisons has precluded such an evaluation.

**METHODS:** We evaluated patterns of among- and within-species variation in HP load size and diversity in 19 species across three distinct plant communities. We assessed the importance of phenotypic specialization (floral phenotype), ecological specialization (contemporary visitor assemblage), and conspecific flower density as determinants of among-species variation. We present hypotheses for different accrual patterns of HP within species based on the evenness and quality of floral visitors and evaluated these by characterizing the relationship between conspecific pollen (CP) and HP receipt.

**KEY RESULTS:** We found that within-species variation in HP receipt was greater than among-species and among-communities variation. Among species, ecological generalization emerged as the strongest driver of variation in HP receipt irrespective of phenotypic specialization. Within-species variation in HP load size and diversity was predicted most often from two CP-HP relationships (linear or exponentially decreasing), suggesting that two distinct types of plant-pollinator interactions prevail.

**CONCLUSIONS:** Our results give important insights into the potential drivers of among- and within-species variation in HP receipt. They also highlight the value of explorations of patterns at the intraspecific level, which can ultimately shed light on plant-pollinator-mediated selection in diverse plant communities.

**KEY WORDS** co-flowering community; diversity; ecological generalization; floral specialization; heterospecific pollen; floral visitor assemblage; pollinator sharing

The movement of pollinators between plants in multispecies plant communities can result in the transfer of pollen from heterospecifics as well as conspecifics (e.g., Feinsinger et al., 1986; Montgomery

and Rathcke, 2012; Fang and Huang, 2013). However, the likelihood of heterospecific pollen (hereafter, HP) receipt varies widely among species. For instance, a review of published studies (Ashman and Arceo-Gómez, 2013) revealed variation among species in both the percentage of flowers that received any HP (2–100%), as well as in the average intensity of HP receipt (% HP in total stigmatic pollen load: 0.1–80%). Furthermore, Fang and Huang (2013) uncovered variation among species within a single community not only in HP load size, but also in the number and identity of HP donors. Some species received large multispecies loads, while others received small monospecific loads of HP (Fang and Huang, 2013). Variation in HP receipt among individuals within a single species can also be extensive (e.g., 1–95% of total load in *Mimulus*

<sup>1</sup> Manuscript received 6 April 2015; revision accepted 9 July 2015.

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doi:10.3732/ajb.1500155

*guttatus*; G. Arceo-Gómez and T.-L. Ashman, unpublished data), but variation at this level has received much less attention. In fact, a systematic comparison of all aspects of HP receipt (e.g., mean and variance of HP load size and diversity) among and within species has not been reported for any community. Characterization of the pattern of variation at inter- and intraspecific levels as well as identifying the factors that contribute to variation at both levels are crucial for gaining a complete understanding of the role of HP transfer in floral evolution and species coexistence in diverse plant communities.

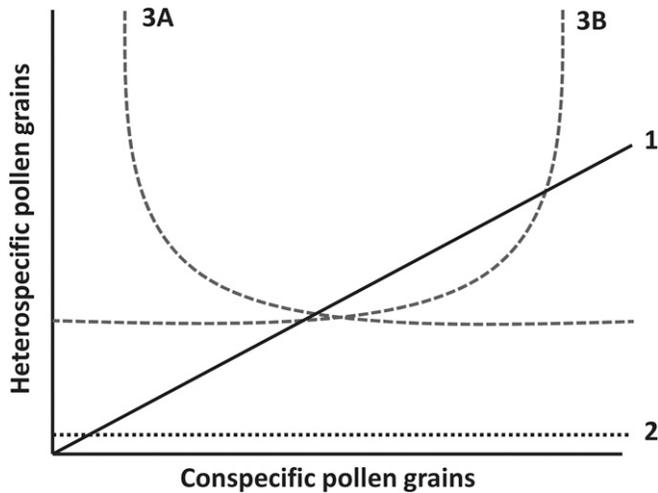
Phenotypic specialization is thought to reflect past selection to maximize conspecific pollen (hereafter, CP) receipt and export while minimizing HP transfer (Rathcke, 1983; Waser, 1983; Armbruster, 1995; Waser et al., 1996; Muchhala and Potts, 2007; Pauw, 2013). Flower shape (e.g., symmetry), in particular, can restrict visitors by body size, tongue length and/or learning capabilities leading to predictions of lower HP receipt by zygomorphic flowers than actinomorphic ones (Faegri and van der Pijl, 1979; Feinsinger et al., 1986; Waser, 1986; McLernon et al., 1996). Irrespective of floral symmetry, however, species with large flowers, long life times, and/or large exerted stigmas are accessible to a wide array of pollinators (see below) and thus are expected to receive large and possibly diverse loads of HP (Montgomery and Rathcke, 2012; Ashman and Arceo-Gómez, 2013; Fang and Huang, 2013; Huang and Shi, 2013). In addition, species with such floral features (i.e., actinomorphic and large, long-lived flowers) would be predicted to also exhibit less variation among plants, as every flower would receive at least some HP over their life. In contrast, species with more restrictive floral phenotypes and small, short-lived flowers are expected to receive, on average, smaller and less diverse HP loads, but may have higher variance among conspecifics as HP delivery is predicted to be more stochastic. These predictions for HP load size among species have been partially borne out (McLernon et al., 1996; Eaton et al., 2012; Montgomery and Rathcke, 2012; Fang and Huang, 2013; Huang and Shi, 2013), but those for variance among and within species have yet to be explored.

Separate from the evolutionary state of specialization reflected in floral phenotype addressed above, the ecological state of specialization, that is, the contemporary community of floral visitors (sensu Feinsinger et al., 1986; Waser et al., 1996; Fenster et al., 2004) is expected to be an important determinant of the among-species variation in amount and diversity of HP deposited on stigmas (i.e., alpha diversity: number of pollen morphs observed on a stigma). From this perspective, a plant species can range from being highly specialized, (i.e., visited by only a single taxon; Pellmyr et al., 1996; Fleming and Holland, 1998; Weiblen, 2004) to widely generalized (e.g., visited by up to 70 different taxa; Waser et al., 1996; Olesen and Jordano, 2002; Vazquez and Aizen, 2003; Alarcón et al., 2008; Bosch et al., 2009). As a consequence, plant species that host a wider array of visitors (that may also visit other plant species) are predicted to receive larger, more diverse loads of HP with low variance among flowers (in load size and number of species in the HP load) than species with less-diverse pollinator assemblages, all else being equal (e.g., pollen placement). This relationship between HP receipt and pollinator diversity has been explored indirectly via the presumed association between ecological specialization and phenotypic specialization (e.g., generalized pollination-actinomorphic flowers vs. specialized pollination-zygomorphic flowers; McLernon et al., 1996). A direct test of the effect of ecological specialization on HP receipt, however, requires measures of the contemporary

community of floral visitors. Simultaneous tests of floral morphology and the degree of ecological specialization are needed to evaluate their relative importance in determining patterns of HP receipt across species (see Fang and Huang, 2013). Furthermore, for species visited by generalist pollinators, the floral neighborhood can also be important in influencing patterns of HP receipt (Rathcke, 1983; Feinsinger et al., 1986). For instance, the local abundance of conspecifics relative to heterospecifics may affect HP receipt, as rare species are expected to receive more (and more diverse) HP than common species (Rathcke, 1983; Vamosi et al., 2006; Alonso et al., 2010). To date, only a small handful of comparative studies have addressed these factors (see McLernon et al., 1996; Fang and Huang, 2013), and as of yet, no study has incorporated all three.

Additional insight into the dynamics of HP transfer in natural communities can be gained from more detailed analysis of intraspecific variation. For instance, the evenness of pollinator quality within the assemblage could affect the pattern and variance in HP receipt among conspecific individuals. Specifically, because flower-visiting taxa (or functional groups) have differences in body size, flight distances, and floral constancy, they can vary in the size and purity (i.e., CP vs. HP) of the pollen load they deposit (“pollination quality”; Herrera, 1987). For instance, Hymenoptera deposited more CP than Lepidoptera and Diptera in Mediterranean *Lavandula latifolia* (Herrera, 1987) and bats deposited larger amounts of HP relative to hummingbirds and hawkmoths in tropical *Aphelandra acanthus* (Muchhala et al., 2009). Thus, low quality floral visitors are those that deposit little CP but large amounts of HP, whereas high quality visitors deposit a lot of CP but little or no HP (Herrera, 1987). As a result, within an assemblage, the evenness of flower-visiting taxa (or functional groups) that differ in their quality may be an important determinant of the distribution of HP among flowers. This hypothesis can be assessed for a single plant species by estimating the pollination quality of each visiting group within its assemblage (e.g., Herrera, 1987; Muchhala et al., 2009), but such an approach is prohibitive for comparing among multiple plant species. Here we propose that the pattern of CP and HP receipt among plants within a species will reflect the joint effect of quality and evenness of their floral visitor assemblage and that these CP-HP relationships can be readily compared among species.

Specifically, we envision three ways HP receipt could covary with CP receipt in flowers within a species. In the first, HP receipt increases monotonically as CP increases (type 1 in Fig. 1). This relationship would occur when both CP and HP grains are deposited with each pollinator visit, as expected when an evenly diverse assemblage of pollinators of similar quality visit flowers. Little variation in HP load size is expected among flowers and HP loads should be diverse because floral visitors are also visiting many other plant species (i.e., generalists; Neiland and Wilcock, 1999). The second possibility is that the delivery of HP is independent of the delivery of CP, leading to no relationship between CP and HP receipt across conspecifics (type 2 in Fig. 1). This pattern could occur when a species is visited by only one or very few high quality pollinators that deliver almost pure CP loads (e.g., specialists), and thus HP receipt occurs only as a result of pollinator “mistakes” (e.g., Wang et al., 2013), which lead to unpredictable patterns of HP deposition. A third alternative is that HP receipt decreases or increases exponentially as CP receipt increases (type 3A and 3B in Fig. 1). These patterns are expected to occur when a species is visited by a highly



**FIGURE 1** Predicted intraspecific relationships between conspecific (CP) and heterospecific pollen (HP) receipt per flower derived from variation in the evenness and quality of the floral visitor assemblage (see introduction). Four relationships are hypothesized: (1) HP receipt increases linearly with CP receipt (solid line); (2) HP varies independently of CP receipt, and thus no relationship exists between HP and CP receipt (dotted line); (3A) HP decreases or (3B) increases exponentially with increasing CP receipt (dashed lines). Note that curve 2 is shown below curves 3A and 3B for visual clarity and not because HP is predicted to be lower.

uneven pollinator assemblage that is composed of one or few frequent, high-quality pollinators and also several less frequent, low-quality pollinators (e.g., Gómez et al., 2010). We note that these predictions rest on the assumption that high-quality pollinators also tend to visit flowers more frequently than low quality ones—a pattern often observed in natural communities (e.g., Motten et al., 1981; Herrera, 1987; Sahli and Conner, 2006; Gómez, 2000; Gómez et al., 2007, 2010). Thus, in species with exponential patterns of CP-HP receipt, HP would be deposited mostly during visits by low-quality pollinators that are less frequent and exhibit low constancy. As a result, the HP load varies with CP (either positively or negatively) depending on the CP load size deposited by the more frequent high-quality pollinators in the assemblage. For instance, if high-quality pollinators deliver large and pure CP loads, whereas HP is delivered along with few CP grains by low-quality pollinators, then HP would decrease exponentially as CP receipt increases (type 3A in Fig. 1). Alternatively, if numerous high-quality pollinators deliver small but pure CP loads and infrequent low-quality pollinators deliver large, mixed pollen loads then HP will increase exponentially with CP (type 3B in Fig. 1). Furthermore, in species with exponential patterns of CP-HP receipt (increasing and decreasing), the unevenness of pollinator quality in the assemblage would lead to an unequal distribution of HP loads across flowers,

i.e., HP will be aggregated in only a few flowers as a result of infrequent visits by low quality pollinators, and there will be high within-species variance in HP load size. Moreover, the pollen species diversity of HP loads for plant species with type 3 CP-HP relationships is predicted to be lower than that of plant species with a linear CP-HP relationship (type 1) because the number of pollen species deposited during infrequent and haphazard visitation events by low-quality pollinators is expected to be lower than when an even assemblage of generalist pollinators are engaged in regular, consistent co-transport of CP and HP. These predictions can be tested by fitting curves to natural variation in CP and HP in flowers within species and comparing the evenness of pollinator assemblages and diversity of HP loads between groups defined by HP-CP relationships.

Accordingly, the aim of this study was to evaluate patterns of among- and within-species variation in HP receipt (load size and diversity [alpha diversity]) and to identify potential drivers of such variation. First, we assessed the relative importance of phenotypic and ecological (i.e., contemporary floral visitor assemblage) specialization, as well as conspecific flower density as determinants of among-species differences in mean and variance of HP load size and diversity received by flowers. Second, we evaluated patterns of within-species variation by characterizing CP-HP relationships (Fig. 1) and the diversity in the HP load and determined whether differences among species are related to the evenness of their floral visitor assemblage. We conducted these analyses with 19 plant species from three geographically distinct coflowering communities and thereby assessed the generality of these patterns across a wide range of ecological contexts.

**MATERIALS AND METHODS**

**Study systems**—Heterospecific pollen receipt was studied in 19 species sampled across three geographically and ecologically distinct coflowering plant communities: serpentine seeps of California, United States (SS), dolomite outcrops in Andalusia, Spain (DO) and dry scrublands in Yucatan, Mexico (DS) (Table 1). Andalusia and California have a Mediterranean climate, while the Yucatan is considered a tropical dry region. In California, the study was conducted in the serpentine seeps at the McLaughlin Natural Reserve where plant species are adapted to serpentine soils and restricted spatially and temporally by water availability (Freestone and Inouye, 2006). In Andalusia, the study took place at the Cazorla Natural Park where plants inhabit sandy limestone-dolomitic outcrops within a mountainous landscape. In Mexico, the study was conducted in the north coast of the Yucatan Peninsula in flat areas surrounded by secondary vegetation that grows on karstic soils. While each community has a unique evolutionary history, all three are seasonally flowering, species-rich communities (Table 1) dominated by small woody or herbaceous perennials and annuals

**TABLE 1.** Location, community type (code), flowering period, number of focal species, total coflowering species richness, flower visitor functional groups observed and number of pollen morphotypes identified at each location.

Location	Community	Flowering period sampled	No. of focal species	No. of insect pollinated coflowering species	No. of flower visitor functional groups	No. of pollen morphotypes
California	Serpentine seeps (SS)	June–July 2010	6	50	11	20
Andalusia	Dolomite outcrops (DO)	May–June 2010	9	67	11	32
Yucatan	Dry scrublands (DS)	July–September 2011	4	38	10	16

(Estrada-Loera, 1991; Médail and Quézel, 1997, 1999; Safford et al., 2005; Freestone and Inouye, 2006; Alonso et al., 2013) that support many functional groups of insect flower visitors (e.g., Herrera et al., 2006; Campos-Navarrete et al., 2013; Koski et al., 2015). Thus, these communities reflect ecologically and evolutionarily independent replicates of similarly diverse plant–pollinator communities (Table 1). In the Mediterranean sites plant species bloom from late spring to early summer while in the tropical sites flowering occurs in response to seasonal rainfall. The study was conducted during peak flowering in 2010 and 2011 from early spring to late summer (Table 1).

Within each community, four to nine insect-pollinated plant species with overlapping flowering times were selected (Table 1). Plant species represented nine families and varied in flower color, size, longevity, mating system, and type of rewards (Table 2, Appendix 1) (Alonso et al., 2013). Both, zygomorphic and actinomorphic species were included from each community and were similarly represented in the data set (10 vs. 9; Appendix 1).

**Data collection—Pollen receipt**—Within each community, an average of 135 wilted flowers (range: 52–222; Table 2) was collected from each species. Every flower was collected from a different individual along 3–5 transects (Appendix 2) that were 20–100 m long depending on the site. Each transect was visited once every 2 weeks during peak flowering, and thus the total number of flowers collected for each species depended on their abundance at each site along the flowering season (Table 2). This sampling scheme allowed us to capture both temporal and spatial variation in pollen receipt for each species. Across the 19 species, styles from a total of 2566 flowers were collected and stored in 70% ethanol until they were stained with decolorized aniline blue and prepared for scoring. Conspecific pollen and HP on stigmas were visualized with the aid of fluorescence microscopy (Kearns and Inouye, 1993). Both CP and HP were counted and summed across all styles within a flower when more than one style was present. A pollen library was

constructed for each community to aid in the identification of pollen to species when possible or to morphotype when not. Hetero-specific pollen was classified based on morphology (size, shape and exine ornamentation). For analysis, HP was pooled into 16 to 32 morphotype categories per community (Table 1). Each morphotype consisted of 1–6 possible species known to coflower (Koski et al., 2015; C. Alonso, T.-L. Ashman, and V. Parra-Tabla, unpublished data). The mean and coefficient of variation (CV) in HP load size (number of HP grains per flower) and diversity (number of HP morphotypes per flower) were calculated for each species (Appendix 1).

**Floral visitors**—The contemporary assemblage of floral visitors was characterized for each species by conducting 3–5 min observations of flowers within fixed plots scaled appropriately for each community (119 2-m<sup>2</sup> plots in SS, 112 4-m<sup>2</sup> plots in DO, and 60 4-m<sup>2</sup> plots in DS). Pollinator censuses were conducted during the period of highest pollinator activity (i.e., between 8:00 and 16:00 hours) twice per plot per week for the duration of the flowering season. A total of 252.5, 32.5, and 183.7 h of observations was conducted at SS, DO, and DS, respectively. During each census, the number of open flowers, and the number and identity of floral visitors on a given species were recorded. Only species that received at least 25 visits were analyzed ( $N = 15$  species; Appendix 1).

Floral visitors are often categorized into functional groups based on their morphology (e.g., body size), energetic requirements, and flight ability, which can determine their foraging and flower-handling behavior, the range of flowers they can visit, as well as their conspecific pollen transfer abilities and thus the type of selection they generate (Fenster et al., 2004; Moretti et al., 2009). In this sense, insects within a pollinator functional group are also expected to be equivalent in their contribution to HP transfer to a given plant species. Members of a functional group are thus more similar to each other in their quality as pollinators than to members of other groups (Zamora, 2000; Fenster et al., 2004; Greenleaf et al., 2007;

**TABLE 2.** Nineteen study species (species code used in Fig. 2; for complete taxonomic names see Appendix 1), plant family (family codes), community type, and sample size ( $N$ ). For each, the evenness of flower visiting fauna, within-species variance (CV) in HP load size (pollen grains), and mean number of HP morphotypes received is given, as well as the type of CP-HP relationship (described in Fig. 1) observed. For each CP-HP regression, the coefficient ( $b$ ) and its significance are given. A dash denotes data not available.

Community	Family (code)	Species code	$N$	Pollinator evenness	CV of HP load size	Mean number of HP types	HP-CP pattern	$b$	$P$
SS	Plantaginaceae (Pl)	Anc	150	0.51	220.9	1.2	Increasing linearly	0.04	0.0007
SS	Ranunculaceae (Ra)	Deu	150	0.68	102.9	2.7	Increasing linearly	0.32	0.0001
SS	Phrymaceae (Ph)	Mig	151	0.19	237.2	2.09	Decreasing exponential	-0.0015	0.0001
SS	Phrymaceae (Ph)	Min	151	0.35	318.8	1.3	Decreasing exponential	-0.002	0.0004
SS	Liliaceae (Li)	Trp	115	0.53	137.4	3.8	Increasing linearly	0.99	0.0001
SS	Liliaceae (Li)	Ziv	151	0.52	156.5	1.8	Increasing linearly	0.032	0.03
DO	Cistaceae (Ci)	Fub	113	—	187.9	1.2	Decreasing exponential	-0.0009	0.0001
DO	Cistaceae (Ci)	Fup	63	0.6	260.4	1.5	Decreasing exponential	-0.004	0.008
DO	Cistaceae (Ci)	Hcr	90	0.23	395.8	1.4	Decreasing exponential	-0.006	0.003
DO	Cistaceae (Ci)	Hci	129	—	612.1	0.27	Decreasing exponential	-0.005	0.001
DO	Lamiaceae (La)	Sii	123	0.37	580.6	0.03	No pattern	0.0001	0.86
DO	Caryophyllaceae (Ca)	Sco	133	—	349.2	2.3	Increasing exponential	0.005	0.002
DO	Caryophyllaceae (Ca)	Spl	200	—	338.8	3.1	Increasing linearly	0.09	0.01
DO	Lamiaceae (La)	Tpo	222	0.31	285.7	0.29	Decreasing exponential	-0.004	0.0001
DO	Lamiaceae (La)	Tho	118	0.23	349.7	0.14	Decreasing exponential	-3.6	0.0001
DS	Plantaginaceae (Pl)	Ang	52	0.31	413.4	0.28	Increasing linearly	0.17	0.0004
DS	Malvaceae (Ma)	Cie	212	0.19	300.4	0.77	Increasing linearly	0.05	0.0001
DS	Lythraceae (Ly)	Cup	97	0.4	256.2	0.27	Increasing exponential	0.01	0.002
DS	Malvaceae (Ma)	Sid	148	0.43	246.3	0.25	Increasing exponential	0.01	0.001

Geslin et al., 2013; Rosas-Guerrero et al., 2014; Koski et al., 2015), and the presence/absence of a particular group can have important effects on plant fitness (Gómez et al., 2010; Albrecht et al., 2012; Fründ et al., 2013). Thus, in this study, floral visitor diversity was calculated based on 11 behaviorally and functionally defined groups based on their body size, energetic requirements, and foraging/feeding behavior (Appendix 3). These functional groups were present across all three communities and included large solitary bees, small solitary bees, large social bees, beetles, bee flies, large syrphid flies, small syrphid flies, other flies, butterflies, walking insects (e.g., ants), and wasps (Appendix 3). Simpson's reciprocal diversity index ( $1/D$ ) and evenness were calculated for each study species using data on number of visits by these functional groups. Simpson's reciprocal index ( $1/D$ ) was chosen because it is an abundance weighted diversity estimate [ $1/D = 1/\sum (n/N)^2$ , where  $n$  = total number of organisms in a particular functional group, and  $N$  = total number of organisms in all functional groups], that is very robust even with small sample sizes and thus is a reliable measure of diversity (Magurran, 2004). Rarefaction analyses demonstrated that our sampling effort was sufficient to adequately characterize the diversity of pollinator functional groups visiting plant species as we were able to capture between 80–98% of all possible plant–pollinator interactions (Koski et al., 2015; C. Alonso et al., unpublished data). The diversity of pollinator functional groups was not correlated with the total number of visits observed for a given species ( $r = 0.09$ ,  $P = 0.7$ ).

Simpson's evenness was calculated by dividing Simpson's reciprocal index by the total number of pollinator functional groups in the sample (Magurran, 2004). Thus, evenness varies from 0 (completely uneven) to 1 (complete evenness).

**Conspecific density**—Conspecific flower density for each focal plant species was determined from the records of the number of open flowers per plot during each floral visitor census. To produce a single value per species of local conspecific density (flowers/m<sup>2</sup>) flower densities were averaged across the season for every plot where that species flowered. This estimate of local conspecific flower density is correlated with its global floral abundance in the community ( $r = 0.99$ ;  $P = 0.001$ ,  $N = 19$ ).

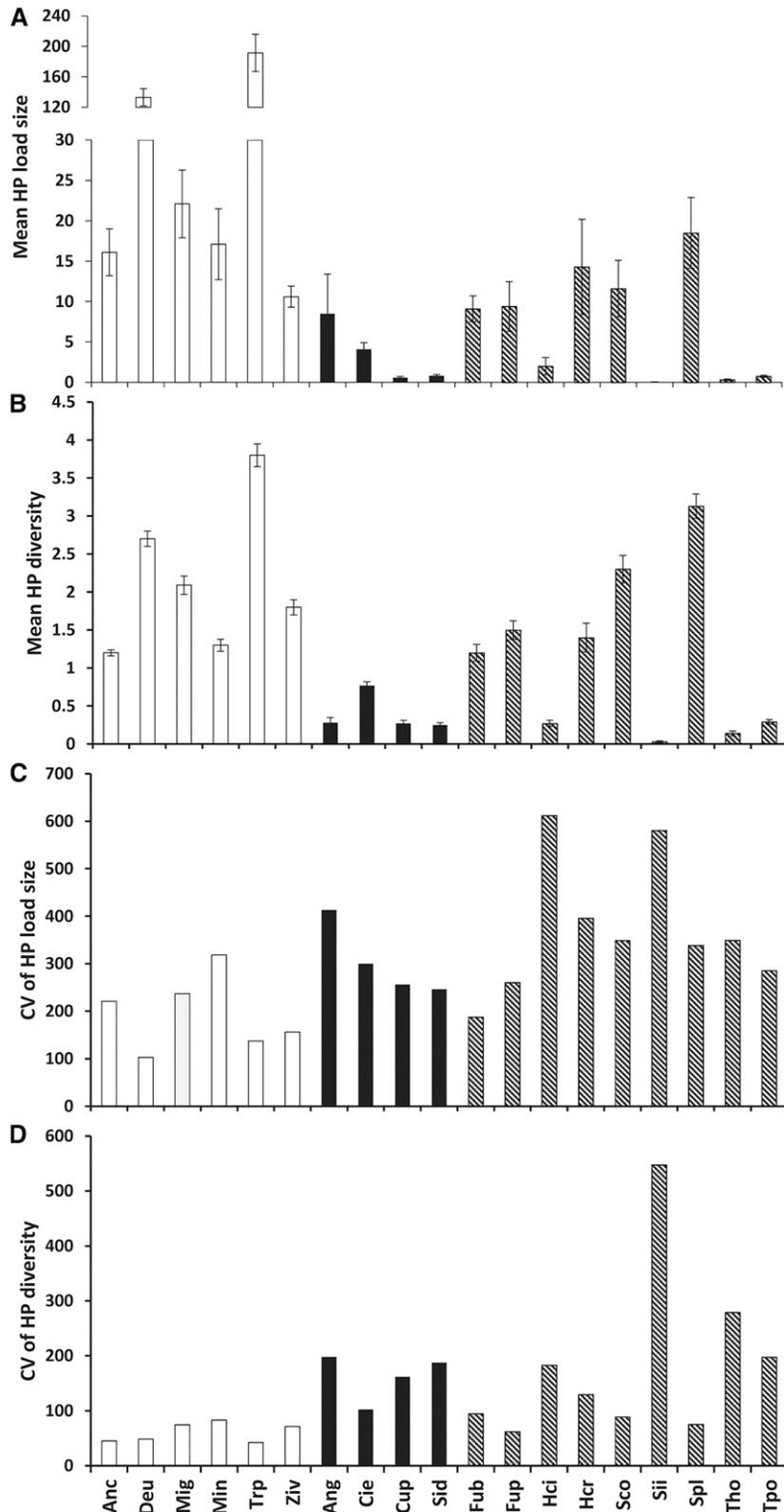
**Flower traits**—Flower biomass was used as a proxy of flower size. For each species, we collected a minimum of 15 flowers, and these were dried in bulk at 60°C for 24 h and then weighed to the nearest 0.0001 g. Total dry mass was then divided by the total number of flowers to estimate average flower biomass for each species. Flower longevity was estimated by recording the number of days a flower remained open (from anthesis to senescence) on a minimum of five individuals (excluded from pollinators) per species during peak flowering in each community. The average flower size and longevity for each species was used in analyses. Floral shape was scored as zygomorphic (bilateral symmetry) or actinomorphic (radial symmetry).

**Data analyses**—*Sources of variation in HP receipt*—To evaluate the pattern of variation in size (number of HP grains) and diversity (number of HP types) of the HP load per flower, we conducted nested mixed effects ANOVAs (proc mixed; SAS Institute, 2010). To account for the potential influence of shared evolutionary history on species patterns of HP receipt, family and species (nested within family) were treated as random effects, while community

was treated as a fixed effect. We then used this same model to partition the total variance in HP load size and diversity among families, species, communities and individuals within species (residual variation; proc varcomp, method = MIVQUE0; SAS Institute, 2010). Load size and diversity of HP types were (square root + 0.5) transformed to meet assumptions of normality of residuals.

*Among-species variation in HP receipt*—Means and CV of HP load size and diversity were calculated for each species and used in among-species regressions to determine the relative importance of ecological specialization (contemporary floral visitor diversity), conspecific flower density, and phenotypic specialization (flower size, longevity and symmetry) in explaining interspecific variation in HP receipt. Given the minor, but significant, effect of community on HP load size and HP diversity (see results), the effect of community was removed using a single effect linear model (proc glm; SAS Institute, 2010) and using the residuals in subsequent regression analyses across all species regardless of their community (e.g., Jakob et al., 1996; Essenberg, 2013). There was no effect of community on any of the independent variables studied (all  $P > 0.3$ ), so raw data were used in the regression analyses. Standardized regression coefficients were calculated for each variable (each observation minus the mean and divided by the variance) to facilitate comparisons among independent variables. To test for an effect of continuous variation in phenotypic specialization (flower size and longevity), ecological specialization and conspecific flower density, multiple regressions were conducted. To test for an effect of categorical variation in phenotypic specialization (flower symmetry: actinomorphic vs. zygomorphic) and its potential interaction with floral visitor diversity and conspecific density an ANCOVA was run (proc glm; SAS Institute, 2010). When interactions between flower symmetry and visitor diversity or conspecific density were nonsignificant ( $P > 0.5$ ), they were removed from the models. Heterospecific pollen load size, diversity of HP types (square root + 0.5), and conspecific flower density (log 10) were transformed to improve the distribution of residuals. Insect visitation and flower size data were not available for four and one species respectively, (Appendix 1), so the final data set for the analysis of species-specific traits on HP receipt included 14 species.

*Within-species variation in HP receipt*—To characterize within-species relationships between HP and CP receipt (Fig. 1), linear and nonlinear regressions between the amount of CP and HP received per flower were performed separately for each species. First, we tested for the significance of both linear (proc reg; SAS Institute, 2010) and nonlinear relationships between CP and HP receipt. The nonlinearity of the relationship was tested with the inclusion of a nonparametric component (spline) using a generalized additive model with a cubic spline and a Poisson error distribution (proc gam; SAS Institute, 2010). If only a significant linear relationship was found, then the species was classified as type 1. If both (linear and nonlinear) were found to be nonsignificant then the species was categorized as type 2 (amount of HP received independent of the amount of CP). Only when the nonlinear relationship was found significant was an exponential model ( $HP = a \times (\exp[b_1 \times CP])$ ) then fit to the data (proc nlin; SAS Institute, 2010). Species with a significant negative  $b$  have an exponentially decreasing CP-HP relationship (type 3A), while those with positive  $b$  have an exponentially increasing CP-HP relationship (type 3B). If both linear and nonlinear relationships were found to be significant, then we assessed the



**FIGURE 2** (A, B) Mean ( $\pm$ SE) and (C, D) coefficient of variation (CV) for heterospecific pollen (HP) load size (number of grains per flower) and HP diversity (number of HP morphotypes per flower) for each of the 19 studied plant species. Species were sampled in three plant communities: serpentine seeps (open bars), dry scrubland (solid bars), and dolomite outcrops (dashed bars; Table 1). Species codes follow names in Table 2. Note the broken axis in panel A.

fit of the models by comparing adjusted  $R^2$ s and the model with the best fit was selected (larger  $R^2$  values). Pseudo  $R^2$  for nonlinear models was estimated as  $1 - (\text{Error sum of squares}/\text{Total sum of squares} [\text{corrected}])$  (Statistical Consulting Group, 2015). Finally, we performed linear models to evaluate whether species with different patterns of CP-HP receipt (linear vs. nonlinear: type 1 vs. 3A and 3B; see results) also differed significantly in evenness of flower-visiting fauna, CV of HP load size or total HP diversity received. These models were based upon the residuals from a one-way ANOVA where community was the predictor of each response variable.

## RESULTS

**Sources of variation in HP receipt**—We found extensive variation among species in mean HP load size (mean  $\pm$  SE:  $24.7 \pm 11.4$ ; range: 0.04–191.5 pollen grains) and HP diversity ( $1.3 \pm 0.25$ ; 0.03–3.8 morphotypes) per flower as well as in their respective CVs ( $302.6 \pm 30.5$ ; 102.9–12.1 and  $140.6 \pm 27.1$ ; 42.1–547.6 for HP load size and diversity respectively; Fig. 2; Appendix 1).

Variation among individuals within a species (residual variation) accounted for the highest proportion of the total variation in HP load size and HP diversity (46 and 50% respectively). Nevertheless, the contribution of species-level variation to total variation for both response variables was also substantial (28 and 10%, respectively). Variation among communities explained 23 and 7%, and plant family explained 3 and 33% of the total variation in HP load size and HP diversity, respectively.

The ANOVA results showed that the average HP load size and HP diversity received per flower was significantly different among species ( $Z = 1.86$ ,  $P < 0.03$  and  $Z = 2.18$ ,  $P < 0.01$ , respectively). Heterospecific pollen load size and diversity also varied significantly among communities ( $F_{2, 2547} = 5.77$ ,  $P = 0.003$  and  $F_{2, 2547} = 4.28$ ,  $P = 0.01$ , respectively), while family had no effect in either response variable (both  $Z < 1.4$ ,  $P > 0.05$ ).

**Among-species variation in HP receipt**—There was wide variation in floral visitor diversity ( $2.02 \pm 0.25$ ; 1.05–4.09), conspecific flower density ( $19.7 \pm 1.25$ ; 0.6–217.2 flowers/m<sup>2</sup>), flower longevity ( $3.8 \pm 0.79$ ; 0.5–12 d), and flower biomass ( $0.008 \pm 0.001$ ; 0.0006–0.29 g) among species providing ample discriminatory ability in regressions even with a small number of species (Appendix 1).

Among species, mean HP load size increased significantly with increasing floral visitor diversity and flower biomass (Table 3A). Floral visitor diversity was the only significant factor affecting HP load diversity, and this effect was positive (Table 3B). Conspecific flower density and flower longevity did not significantly affect either aspect of HP receipt (Table 3A, B).

**TABLE 3.** Multiple regressions for the effects of flower biomass, longevity, floral visitor diversity, and conspecific flower density on mean and variance (CV) of HP load size (number of grains) and diversity (number of morphotypes) ( $N = 14$  species). The coefficient of determination ( $R^2$ ) and significance are given for each model along with the standardized regression coefficients ( $\beta$ ) for each independent variable. Significant models and regression coefficients ( $P < 0.05$ ) are denoted in bold face.

Variables		Model			
Dependent	Independent	$\beta$	$P$	$R^2$	$P$
A) Mean HP load size	Flower biomass	0.44	<b>0.05</b>	0.78	<b>0.005</b>
	Flower longevity	0.01	>0.9		
	Flower visitor diversity	0.54	<b>0.02</b>		
	Conspecific density	-0.01	>0.9		
B) Mean HP diversity	Flower biomass	0.29	0.12	0.84	<b>0.001</b>
	Flower longevity	-0.23	0.14		
	Flower visitor diversity	0.53	<b>0.01</b>		
	Conspecific density	-0.23	0.2		
C) CV of HP load size	Flower biomass	0.11	>0.7	0.42	0.24
	Flower longevity	0.24	>0.4		
	Flower visitor diversity	-0.64	0.07		
	Conspecific density	0.02	>0.9		
D) CV of HP diversity	Flower biomass	-0.14	>0.6	0.57	0.07
	Flower longevity	0.56	0.04		
	Flower visitor diversity	-0.16	>0.5		
	Conspecific density	0.1	>0.4		

Although there was substantial variation among species in the CV of HP load size and HP diversity (see *Sources of variation in HP receipt*), none of the factors we measured significantly explained this variation (Table 3C, D).

When flower symmetry was included in a separate model to account for categorical variation in floral phenotype (see data analysis above), the results did not change. That is, the strong effects of floral visitor diversity and the nonsignificant effect of conspecific density on HP load size ( $F_{1,9} = 14.5, P = 0.004; F_{1,11} = 0.4, P > 0.5$ , respectively) and HP diversity ( $F_{1,9} = 9.96, P = 0.01; F_{1,9} = 0.8, P > 0.4$ ) remained. There were no significant interactions of flower symmetry with either continuous variable ( $F_{1,9} = 0.04-1.3$ ; all  $P > 0.3$ ). And even though average HP load size and HP diversity were slightly elevated in actinomorphic flowers compared to zygomorphic ones, these were not statistically significant differences (HP load size:  $27.2 \pm 18.3$  vs.  $22.1 \pm 14.2$  grains;  $F_{1,11} = 0.18, P > 0.6$ ; HP diversity:  $1.6 \pm 0.3$  vs.  $0.9 \pm 0.3$ ;  $F_{1,11} = 1.5, P = 0.24$ ).

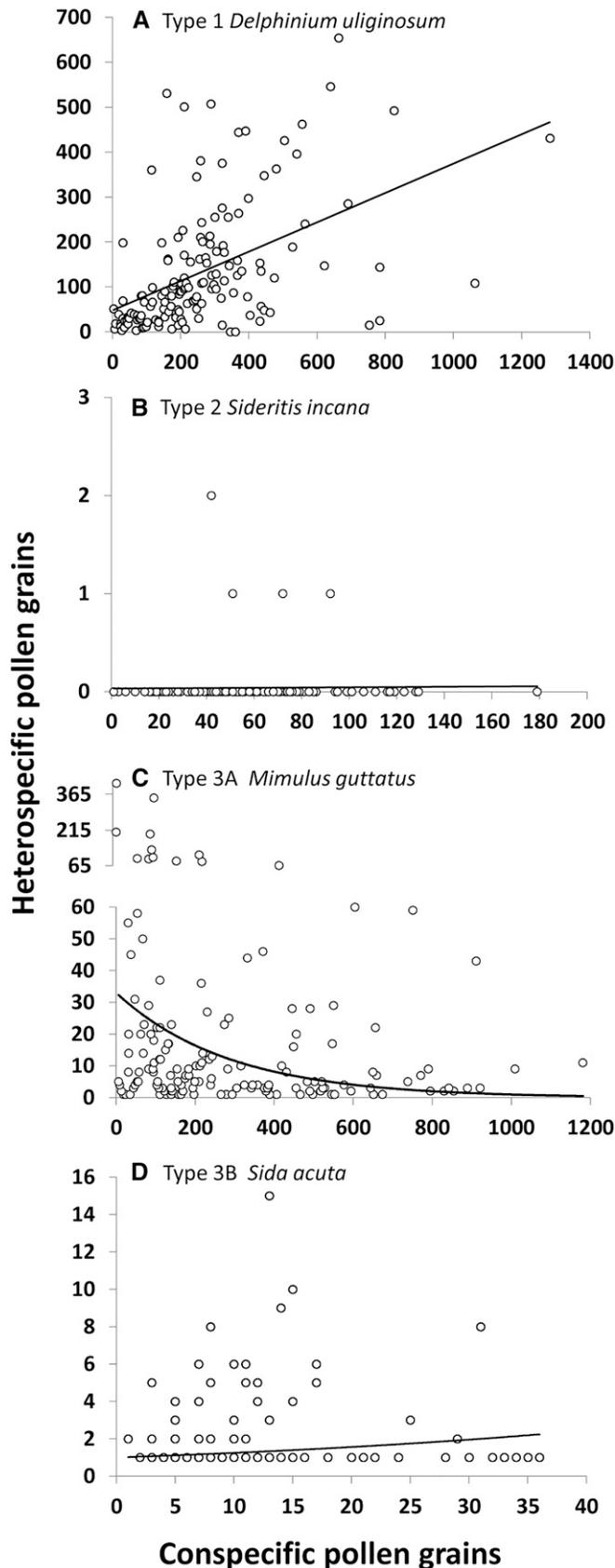
**Within-species variation in HP receipt**—Of the 19 species evaluated, seven exhibited significant linear relationships between the amount of CP and HP received (type 1 in Fig. 1; Fig. 3A for an example), eight showed an exponentially decreasing relationship (type 3A in Fig. 1; Fig. 3C) and three showed an exponentially increasing relationship (type 3B in Fig. 1; Fig. 3D; Table 2). Only in one species did HP vary independently of CP (type 2 in Fig. 1; Fig. 3B; Table 2). Furthermore, differences in CP-HP patterns (linear type 1 vs. exponential type 3A and 3B) reflected differences in HP load size, HP diversity, and pollinator evenness in the predicted directions, although none of these relationships were statistically significant (HP diversity:  $1.8 \pm 0.3$  vs.  $0.9 \pm 0.3$ ;  $F_{1,16} = 3.21, P = 0.09$ ; CV in HP load size:  $291 \pm 36$  vs.  $270 \pm 42$ ;  $F_{1,16} = 0.11, P > 0.7$ ; pollinator evenness:  $0.44 \pm 0.08$  vs.  $0.34 \pm 0.06$ ;  $F_{1,16} = 1.16, P > 0.4$ ). There was also no association between flower symmetry and CP-HP relationship type ( $\chi^2_{16} = 0.01, P > 0.9$ ).

**DISCUSSION**

Wide variation in mean HP load size and HP diversity in naturally pollinated flowers was revealed in this detailed comparison of 19 species across three plant communities. Diversity of the contemporary flower-visiting community emerged as a strong and consistent driver of variation in HP receipt among species, more so than evolutionary degree of specialization, as represented by floral phenotype (symmetry, biomass, and longevity). Furthermore, within-species variation in HP load size and HP diversity could be associated with patterns of CP receipt, and two dominant CP-HP relationships emerged (linear or exponentially decreasing) indicating the potential for promising explorations of mechanisms at this level in the future. We discuss these results and their broader ecological implications in detail next.

**Among-species variation in HP receipt**—Plant species identity accounted for more variation in HP load size than family membership suggesting that the processes that influence variation in HP load size are likely independent of common evolutionary history. However, the reverse was observed for HP load diversity where among-family variation was more important. This latter result is consistent with studies that have shown, across many plant-pollinator communities, that pollinator guilds tend to visit closely related plant species (e.g., Vamosi et al., 2014), and thus species within the same plant family are likely to receive similar diversity of HP grains. Furthermore, HP receipt varied across communities but variation among them accounted for less of the total variation observed than did variation among species and individuals in both HP load size and HP diversity, suggesting that underlying processes that influence HP receipt may operate across a wide range of ecological and evolutionary contexts. Interestingly, each community had species with high mean HP receipt and species with very low HP receipt (Fig. 2). For example, in the serpentine seeps, *Delphinium uliginosum* and *Triteleia peduncularis* received hundreds of HP grains—10 times the HP per flower received by *Zigadenus venenosus*, and in the dolomite outcrops *Silene lasiostyla* received 20 times that of *Thymus orospedanus* or *Sideritis incana* (Fig. 2; Appendix 1). Similar dramatic differences have been observed between species within alpine (Fang and Huang, 2013), prairie (Montgomery and Rathcke, 2012), and old-field communities (McLernon et al., 1996), suggesting that avoidance of HP may not be the only evolutionary stable strategy for coexistence. That is, there may be two evolutionary strategies: selection for avoidance of HP on one hand and selection for tolerance of the effects of HP receipt on the other that could contribute to species coexistence (Ashman and Arceo-Gómez, 2013). Studies that link natural patterns of HP receipt to their causes (discussed later) as well as fitness costs are needed to test these ideas (Ashman and Arceo-Gómez, 2013).

There was also substantial among-species variation in the diversity of HP loads. However, given our use of morphotypes (which each could represent 1–6 species), these data most likely represent underestimates of the diversity of interactions. In fact, when species have been identified, up to 15 species of HP can occur on one flower (Bartomeus et al., 2008; Fang and Huang, 2013). Such extensive HP transfer indicates that pollinator sharing is leading to multispecies interactions on the style for most plants in the community, the consequences of which can be hard to predict based on the effects of individual HP donors alone (Arceo-Gómez and Ashman, 2011). What is clear, however, is that this variation in HP load size and diversity provides a window into understanding the ecological drivers



of HP receipt, and can also help pinpoint where opportunities for natural selection to avoid or tolerate HP receipt lie (see next).

After accounting for community differences, HP load size and HP diversity reflected species-specific floral visitor diversity, more than other measures of ecological context (conspecific density) or phenotypic specialization (floral symmetry, longevity, and biomass). The only other study of HP patterns that directly characterized contemporary floral visitor assemblages also found that more generalized plants tended to receive larger and more diverse loads of HP than specialized ones (Fang and Huang, 2013). Moreover, in our study, the increase in HP load size and HP diversity with increasing floral visitor diversity occurred irrespective of flower symmetry, a finding that supports the notion that phenotypic specialization may not always reflect ecological specialization (Ollerton, 1996). Such a disconnect may also explain patterns of HP receipt contrary to the presumed level of specialization based on symmetry, i.e., higher HP receipt in zygomorphic than actinomorphic flowers found in other studies (McLernon et al., 1996).

Floral traits that defined specialization or restrictiveness, while being quite varied in our data set (Appendix 1), were not strong determinants of variation in any aspect of HP receipt. Only flower biomass affected HP load size among-species, with species with heavier (larger) flowers receiving larger and more diverse HP loads than lighter (smaller) ones. Because we accounted for floral visitor diversity, the effect of flower biomass is not through differences in generalization level but rather may be mediated through enhanced visitation rates (e.g., Galen and Newport, 1987; Conner and Rush, 1996) or larger/more exposed stigmas (Montgomery and Rathcke, 2012; Fang and Huang, 2013). It is also worth pointing out that we measured flower biomass at the level of the whole flower (i.e., including the calyx), potentially including nonattracting portions of the flower, which could have influenced our results. Interestingly, flower symmetry only had a minor effect on mean HP load size, and HP receipt appeared to scale similarly with floral visitor diversity in both actinomorphic and zygomorphic flowers. Furthermore, we did not find any evidence to support the prediction that sparse (rare) species are more likely to receive HP than abundant ones (Rathcke, 1983; McLernon et al., 1996), contrasting with the results of Jakobsson et al. (2009).

Despite the observation of a wide range in CVs of HP load size and HP diversity across species (Appendix 1), none of the predictors we tested could account for this variation (Table 3). The strongest nonsignificant indicator of this variation was flower longevity, possibly reflecting increased visitation rate per flower, and/or increased likelihood of stochastic visitation events by inconstant visitors. But more study is needed to pinpoint the sources of variation in this important aspect of HP receipt.

**Within-species variation in HP receipt**—Within-species variation (CV) in HP load size and HP diversity varied up to 6–10-fold in magnitude across species (Appendix 1), and variance at this level was greater than the combined contributions of family and species

**FIGURE 3** Examples of the four types of relationships between the amount of conspecific (CP) and heterospecific pollen (HP) received on stigmas (described in Fig. 1). (A) Type 1: HP linearly increases with CP in *Delphinium uliginosum*; (B) type 2: HP varies independently with CP in *Sideritis incana*; (C) type 3A: HP exponentially decreases with CP in *Mimulus guttatus*; (D) type 3B: HP exponentially increases with CP in *Sida acuta*. Note the broken axis in panel C.

identity (>46% of the total). Our exploration of within-species variation in HP load size could be predicted from two dominant CP-HP relationships (type 1 vs. 3A in Fig. 1; Table 2) and suggests that two distinct types of plant–pollinator interactions prevail. We found approximately half of the species studied showed significant linear increases in HP with CP (and also tended to have higher diversity of HP types), while most of the remaining species showed exponentially decreasing relationships (and fewer HP types). And while the difference in pollinator evenness between these two groups was in the predicted direction (greater for species with type 1), it was not significant. Nevertheless, the evaluation of CP-HP relationships among flowers provides insight into the genesis of variation in HP receipt that cannot be discerned from means or variances alone, and our comparative data provide first insight into the possible factors responsible for within-species variation in HP receipt. For instance, the fact that evenness alone did not capture the within-species variance in pollen transfer dynamics may suggest that variation in pollinator quality is more important than variation in abundance in influencing deposition dynamics or that variation not captured by our floral visitor functional groups is important. Pollinator quality, in particular, is likely a complex trait that results from not only inconsistency but also pollinator grooming (Thomson, 1986; Holmquist et al., 2012), pollen carryover (Thomson and Plowright, 1980), and pollen loss during transport (Wilcock and Neiland, 2002). Thus, pollinator quality may be difficult to capture with only information on the visitor functional groups defined herein.

Nevertheless, more species-level studies are needed to tease apart the contribution of pollinator evenness from pollinator quality as well as to take into account fine-scale neighborhood variation in flower diversity and abundance. Such work would provide insight into the potential for ongoing evolution of either avoidance (e.g., pollen placement [Armbruster et al., 1994], site of stigma contact [Armbruster et al., 2014], stigma size [Montgomery and Rathcke, 2012]), or tolerance strategies (e.g., stigma secretions [Ashman and Arceo-Gómez, 2013]) in contemporary communities. While it is a daunting task to perform these studies across many species within communities, such studies are needed to understand how traits evolve in multispecies contexts.

## CONCLUSIONS

This study illustrates how widespread the phenomenon of HP receipt is within and across diverse plant communities. In doing so, it highlights ecological generalization as an important determinant of interspecific variation in both the size and diversity of HP load. Indicating that contemporary changes in pollinating fauna (loss of species, shifts in flowering time) will likely translate into changes in the plant–plant interactions on the style (e.g., Brosi and Briggs, 2013). We further confirmed that HP and CP receipt are not independent of each other at the intraspecific level for some species and propose that the shape of such relationship reflects past evolution and may provide the raw material for future evolution to shape avoidance of HP transfer.

## ACKNOWLEDGEMENTS

We thank S. Armbruster, S. Mazer, and an anonymous reviewer for helpful comments on this manuscript. We also thank M. M. Alonso, N. Celaya, C. Molina, L. Salinas-Peba, K. Schuller, and P. Téllez for assistance in the field and laboratory and the Consejería de Medio

Ambiente, Junta de Andalucía, and UC Davis for providing facilities. We acknowledge support by FBBVA through the research project ENDLIMIT (BIOCON08/125) and NSF OISE 0852846 and DEB 1020523 to T.L.A., CONACYT (211982) and SEP to G.A.G., an Ivey McManus Diversity Fellowship and an Andrew Mellon Predoctoral Fellowship (University of Pittsburgh) to G.A.M., U Pittsburgh Brackenridge Fellowship to A.J., and U Pittsburgh Norman H. Horowitz Fellowship to C.K. The Spanish MINECO supports this research through the Severo Ochoa Program for Centers of Excellence to EBD-CSIC (SEV-2012-0262).

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**APPENDIX 1** Information on floral and ecological characters for study plant species in each community (com.; serpentine seeps [SS], dry scrubland [DS], and dolomite outcrops [DO] with family (Fam.) and species (Sp.) codes.

Com.	Fam. code	Species	Sp. code	Floral characters			Ecological characters		HP load size		HP load diversity	
				Mean flower biomass (g)	Floral shape	Mean flower longevity (d)	Flower density per m <sup>2</sup>	Floral visitor diversity (1/D)	Mean	CV	Mean	CV
DO	Ca	<i>Silene lasiostyla</i>	Spl	0.00966	A	4.5	0.6	—	18.5	338.8	3.13	75.2
DO	Ca	<i>Silene colorata</i>	Sco	0.00788	A	—	1.3	—	11.6	349.2	2.3	88.9
DO	Ci	<i>Fumana baetica</i>	Fub	0.00887	A	0.5	1.7	.	9.1	187.9	1.2	94.7
DO	Ci	<i>Fumana paradoxa</i>	Fup	0.00924	A	0.5	3.4	2.4	9.4	260.4	1.5	61.9
DO	Ci	<i>Helianthemum cinereum</i>	Hcr	0.0026	A	2.3	1.9	—	2	612.1	0.27	182.8
DO	Ci	<i>Helianthemum appeninum</i>	Hci	0.01614	A	1	7.6	1.8	14.3	395.8	1.4	129.4
DO	La	<i>Sideritis incana</i>	Sii	0.00418	Z	12	25.9	1.1	0.04	580.6	0.03	547.6
DO	La	<i>Thymus orospedanus</i>	Tpo	0.00069	Z	4	217.2	1.8	0.3	349.7	0.14	279
DO	La	<i>Teucrium polium</i>	Tho	0.00253	Z	10	19.5	1.3	0.75	285.7	0.29	197.7
SS	Li	<i>Triteleia peduncularis</i>	Trp	0.0149	A	4.4	3.6	4.2	191.5	137.4	3.8	42.1
SS	Li	<i>Zigadenus venenosus</i>	Ziv	0.004	A	2.8	5.8	2.5	10.6	156.5	1.8	71.3
DS	Ly	<i>Cuphea gaumeri</i>	Cup	0.002	Z	5	12	2.4	0.59	256.2	0.27	161.8
DS	Ma	<i>Cienfuegosia yucatanensis</i>	Cie	0.02	A	1	2.9	1.3	4.09	300.4	0.77	102.3
DS	Ma	<i>Sida acuta</i>	Sid	0.003	A	0.5	3.5	2.1	0.81	246.3	0.25	187.9
SS	Ph	<i>Mimulus guttatus</i>	Mig	0.00638	Z	3.1	14.5	1.6	22.1	237.2	2.09	74.7
SS	Ph	<i>Mimulus nudatus</i>	Min	0.0022	Z	2.2	11.8	1.1	17.1	318.8	1.3	82.9
SS	Pl	<i>Antirrhinum cornutum</i>	Anc	0.0033	Z	6.2	6	1.5	16.09	220.9	1.2	45.4
DS	Pl	<i>Angelonia angustifolia</i>	Ang	—	Z	1	41.4	1.2	8.5	413.4	0.28	198.2
SS	Ra	<i>Delphinium uliginosum</i>	Deu	0.02975	Z	8.2	10.3	4.1	133.2	102.9	2.7	48.5

Notes: A = actinomorphic, HP = heterospecific pollen, Z = zygomorphic. See Table 2 for family codes. A dash denotes data not available.

**APPENDIX 2** Number and location (GPS coordinates) of transects that were used for flower collection at each of the three study regions.

Region	Transect	Geographic location
Andalusia	1	37°56'N, 02°50'W
	2	37°55'N, 02°50'W
	3	37°56'N, 02°52'W
	4	37°49' N, 02°58'W
	5	37°55' N, 02°53'W
California	1	38°51'N, 122°23'W
	2	38°51'N, 122°24'W
	3	38°51'N, 122°24'W
	4	38°51'N, 122°25'W
	5	38°51'N, 122°27'W
Yucatan	1	21°15'N, 89°35'W
	2	21°17'N, 89°35'W
	3	21°18'N, 89°20'W

**APPENDIX 3** Description of floral visitor functional groups (adapted from Koski et al. [2015]) observed visiting plants in the three studied communities, serpentine seeps (California), dolomite outcrops (Andalusia) and dry scrublands (Yucatan).

Floral visitor functional group	Description
Large solitary bees	Bees with large bodies ( $\geq 10$ mm), solitary, forage on pollen and nectar, pollen carried on hind legs or the underside of abdomen, includes members of Andrenidae and Megachilidae
Small solitary bees	Bees with very small bodies ( $\leq 5$ mm), ectothermic, narrow temperature range of activity, fast flyer, solitary, pollen carried on hind legs, includes members of Halictidae and Apidae
Large social bees	Bees with large bodies ( $\sim 15$ mm), social, efficient forager of pollen and nectar, efficient recruiter, pollen carried on hind legs, includes members of Apidae
Beetles	Pollen eaters, inefficient, mostly ectothermic, narrow daily activity range, short flying range, pollen not carried in specific location on body, includes members of Cleridae and Buprestidae (Coleoptera)
Bee flies	Flies in family Bombyliidae, fast feeder, narrow thermal range, territorial, narrow spectrum of flowers, long mouth parts, pollen not carried on specific location on body
Large syrphid flies	Large flies in family Syrphidae ( $\geq 10$ mm), hoverflies, forage on pollen and nectar, fast flying, specialized digestive system for processing pollen, long foraging time, pollen not carried on specific location on body
Small syrphid flies	Small flies in family Syrphidae ( $\leq 10$ mm), hoverflies, forage on pollen and nectar, fast flying, specialized digestive system for processing pollen, long foraging season, pollen not carried on specific location on body
Other flies	Other flies different from bombyliid and syrphid flies, incidental visitors, small, inefficient, pollen not carried on specific location on body, includes members of Muscidae and Callophoridae
Butterflies	Nectar foragers, long foraging times, visit few flowers per plant, fly long distances, narrow thermal requirements, forage on low concentrated nectar, pollen not carried on specific location on body, includes members of Lepidoptera
Wasp	Carnivorous, secondary flower visitors, some groups visit for nectar, pollen not carried on specific location on body, includes members of Vespidae
Walking insects	Walking insects, pollen not carried on specific location on body, includes members of Formicidae