UNIVERSITY OF CALIFORNIA, SAN DIEGO

Effects of Habitat Fragmentation and Introduced Species on the Structure and Function of Plant-Pollinator Interactions

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Biology

by

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2017

The Dissertation of Keng-Lou James Hung is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2017

DEDICATION

This dissertation is dedicated to my parents, who stopped at nothing to nurture my intellectual curiosity; to my brother, who was my ever-reliable field assistant and encourager; and to my wife, who gave up everything she had to make this venture a reality. This dissertation is as much a product of my hard work as it is your unconditional love, support, and prayers.

This dissertation is also dedicated to the 43,000 bees, wasps, flies, and other insects whose curtailed lives will be forever immortalized in data that will one day be used to secure a brighter future for their kind. You took one for the team; thank you for your sacrifice.

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ACKNOWLEDGMENTS

I would like to thank my many mentors at UCSD, without whose guidance I would still be spinning in circles trying to figure out how to do good science. I aspire to achieve both scientific excellence and amazing work-life balance as you have. I would especially like to thank my advisor, Prof. David Holway, who did everything within his power to provide me the best training possible. You have played an enormous role in the success I enjoy today, and your investment in me will have a lasting impact on the success I will enjoy in the future. Many thanks to the past and current members of the Holway, Nieh, and Kohn Labs at UCSD, who were the fabric of my daily life as a graduate student. You are to me much more than my closest colleagues; I will always see you as family. Many thanks to my "army of undergraduates" at the Holway Lab, who, through the years, were my "boots on the ground" in the field and in the lab. I would have accomplished but a fraction of work without your contributions and cheerful encouragement.

I would like to thank the many taxonomists, naturalists, educators, and administrators who have willingly given up their time and resources to enable me to conduct the most rigorous research and reach the broadest audience possible. You are the reason my work has gained the level of relevance it has today. Many thanks to my peers and colleagues at UCSD, whose friendship has blessed me richly and whose excellence is truly inspirational to me. You are a constant reminder that I am not alone, and not an impostor. Many thanks to the entities who deemed my work worthy of their financial support. I hold myself fully responsible to make good on your investment in my education and research.

I would like to thank my brothers and sisters and mentors and intercessors at Faith Community Church, BEBC, and Agape. “The prayer of a righteous person is powerful and effective”; your prayers, friendship, and care were abundantly sufficient to carry me and my family through all of the challenges we faced in the last six-and-a-half years.

I would like to thank my parents, Chi-Ming Hung and Hui-Mei Chia, my very first research sponsors who provided everything a (very) young scientist could dream of in building his early career. I have embarked on, and completed, this fulfilling journey because of your unconditional love, support, and encouragement to pursue my dreams. Many thanks to my little brother, Keng-Wei Jerry Hung, my very first and all-time favorite field assistant, who never stopped convincing me (and everyone else) that I have what it takes to be successful in science and in life. Many thanks to my son, Christian Ninja Chen Hung, who has brought so much joy and meaning to what I do on a daily basis.

I would like to thank my wife, Hui-San Susan Chen, who gave up everything to let me chase my dreams, and then gave in everything along the way to ensure that I could successfully catch it. The skill, creativity, and discipline I mustered to pursue my PhD pale in comparison to what you accomplish daily for the little family you sustain.

Finally and most importantly, I would like to thank The Lord my God, my Everything. All my accomplishments and glory belong to You.

Chapter 1, in full, has been submitted for publication of the material as it may appear in *PLOS ONE*, 2017, Hung, Keng-Lou J.; Ascher, John S.; Holway, David A., Public Library of Science, 2016. The dissertation author was the primary investigator and author of this manuscript.

Chapter 2, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Ascher, John S.; Davids, Jessica A.; Holway, David A. The dissertation author was the primary investigator and author of this manuscript.

Chapter 3, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Cen, Henry J.; Lee, Adrienne; Holway, David A. The dissertation author was the primary investigator and author of this manuscript.

Chapter 4, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Kingston, Jennifer M.; Albrecht, Matthias; Holway, David A.; Kohn, Joshua R. The dissertation author was the primary investigator and author of this manuscript.

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FIELD OF STUDY

Major Field: Ecology, Behavior, and Evolution

ABSTRACT OF THE DISSERTATION

Effects of Habitat Fragmentation and Introduced Species on the Structure and Function of Plant-Pollinator Interactions

by

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Doctor of Philosophy in Biology

University of California, San Diego, 2017

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Pollination is an important ecosystem function threatened by habitat fragmentation, a globally widespread form of anthropogenic habitat modification. Despite general recognition that habitat fragmentation tends to reduce pollinator species richness, few studies have examined how fragmentation impacts pollinator temporal and functional diversity, or how changes to pollinator diversity may influence the structure and function of plant-pollinator interactions. I compared study plots in coastal sage scrub

habitat fragments and large natural reserves with respect to the temporal, functional, and landscape-level diversity of bees, the most important guild of pollinators in this region. I also documented plant-pollinator interactions in the same study system to examine how loss of pollinator diversity influences the structural properties of plant-pollinator interaction networks. Lastly, I used a meta-analysis to examine the role of the western honey bee, a globally introduced pollinator, in natural habitats worldwide. I found that compared to natural reserves, habitat fragments harbored bee assemblages that are taxonomically and functionally distinct from those in reserves, with consistently reduced taxonomic diversity throughout the study season, lower turnover of bee taxa as the season progresses, and lower functional diversity. However, fragments and reserves harbored similar abundances of bees, and exhibited similar spatial turnover of bee assemblages with respect to both taxonomic and functional diversity. Plant-pollinator interaction networks in fragments exhibited lower interaction selectivity and higher nestedness compared to those in reserves, but networks in fragments and reserves were otherwise structurally similar. Honey bees were numerically dominant across all study sites in my study system, a globally uncommon phenomenon despite the presence of honey bees in most surveyed natural habitats worldwide. The patterns of pollinator diversity loss I documented suggest that large, intact natural reserves are essential for conserving the regional distinctiveness of bee faunas in San Diego as well as the functions they perform. On the other hand, conserving plant-pollinator interactions in habitat fragments appears both possible, thanks to the continued persistence of structurally robust plant-pollinator interaction networks therein, and potentially rewarding, thanks to the high functional and taxonomic beta diversity among fragments.

CHAPTER 1: Urbanization-Induced Habitat Fragmentation Erodes Multiple Components of Temporal Diversity in a Southern California Native Bee Assemblage

ABSTRACT

Despite a large number of ecological studies that document diversity loss resulting from anthropogenic disturbance, surprisingly few consider how disturbance affects temporal patterns of diversity that result from species turnover over time. Temporal dynamics can play an important role in the structure and function of biological assemblages. Here, we investigate the temporal diversity patterns of bee faunas in Southern California coastal sage scrub ecosystems that have been extensively fragmented by urbanization. Using a two-year dataset of 235 bee species (n = 12,036 specimens), we compared 1-ha plots in scrub fragments and scrub reserves with respect to three components of temporal diversity: overall plot-level diversity pooled over time (temporal gamma diversity), diversity at discrete points in time (temporal alpha diversity), and seasonal turnover in assemblage composition (temporal beta diversity). Compared to reserves, fragments harbored bee assemblages with lower species richness and assemblage evenness both when summed across temporal samples (i.e., lower temporal gamma diversity) and at single points in time (i.e., lower temporal alpha diversity). Bee assemblages in fragments also exhibited reduced seasonal turnover (i.e., lower temporal beta diversity). While fragments and reserves did not differ in overall bee abundance, bee abundance in fragments peaked later in the season compared to that in reserves. Our results argue for an increased awareness of temporal diversity patterns, as information about the distinct components of temporal diversity is essential both for characterizing

the assemblage dynamics of seasonal organisms and for identifying potential avenues through which disturbance may impact ecosystem function.

INTRODUCTION

The alteration of natural habitats by human activities is generally acknowledged to reduce the abundance and diversity of organisms (e.g., Vitousek et al. 1997, Laakkonen et al. 2001, Crooks et al. 2004, Winfree et al. 2009, Cardinale et al. 2012). However, our understanding of the consequences of anthropogenic disturbance remains incomplete because most studies that address the effects of disturbance pool or average data over time, ignoring the fact that most biological assemblages exhibit seasonal turnovers in the identity and abundance of species. Given that seasonal variation in diversity and abundance plays an important role in the structure and function of communities (Tylianakis et al. 2005, Fraterrigo and Rusak 2008, Korhonen et al. 2010, Wilsey et al. 2011, Pilosof et al. 2013), the effects of disturbance may be greatly underestimated without explicit consideration of such seasonal dynamics.

To account for how seasonal dynamics influence the response of an assemblage to disturbance, one may separate the assemblage's diversity into temporal gamma, alpha, and beta components in a manner similar to the partitioning of spatial diversity (Whittaker 1972). When diversity is examined in this temporal framework, temporal gamma diversity pertains to data pooled across individual temporal samples from a given locality (Stegen et al. 2013). As such, temporal gamma diversity is equivalent to "site-level diversity," one of the most commonly reported measures of diversity in assemblage- and community-level studies. Temporal alpha diversity pertains to the finest temporal

scale in which sampling is conducted (Lande 1996), providing insight into diversity at discrete points in time and allowing for analyses of temporal trends within a study site. Lastly, temporal beta diversity measures the degree to which individual temporal samples at a study site differ from one another with respect to the composition of taxa present, providing insight into the temporal turnover of the taxa that make up an assemblage (Anderson et al. 2011). While some popular indices of beta diversity are mathematically derived from measures of alpha and gamma diversity (e.g., Whittaker 1972, Lande 1996), recent advancements in the field of statistics have enabled additional measures of beta diversity, such as multivariate dispersion (Anderson et al. 2006), that are mathematically independent of measures of alpha and gamma diversity.

Impacts of anthropogenic disturbance on temporal gamma diversity always result from changes in temporal alpha diversity, beta diversity, or both (Fig. 1-1). Decreases in temporal alpha and beta diversity may be driven by different aspects of disturbance (e.g., Tylianakis et al. 2005), and may have different implications for biological interactions and ecosystem function even if different patterns of temporal alpha and beta diversity loss lead to the same net change in temporal gamma diversity (Fig. 1-1, scenarios 1-3). Trends in temporal alpha and beta diversity may also act in opposition such that temporal gamma diversity remains unchanged in spite of the profound alteration to temporal assemblage structure (Fig. 1-1, scenario 4). Thus, isolating the mechanisms through which disturbance impacts an assemblage requires an examination of all three components of temporal diversity (e.g, Tylianakis et al. 2005). Such approaches may also serve to identify the ecological effects that result from disturbance (e.g., Wilsey et al. 2011, Rafferty et al. 2015).

In this study, we investigated the impacts of urbanization-induced habitat fragmentation on the seasonal dynamics of a diverse native bee (Hymenoptera: Anthophila) assemblage over a two-year period. Bees represent an appropriate taxonomic group for studying how habitat fragmentation affects temporal dynamics because, like many other organisms that occupy seasonal environments, bees exhibit distinct periods of activity that differ among species with respect to both duration and timing of onset (Michener 2007). Previous research has demonstrated that anthropogenic disturbance may differentially impact bee species active in different seasons (Wray and Elle 2015), and that temporal turnover in bee assemblages can contribute to among-habitat differences in site-level bee species richness (Tylianakis et al. 2005). Additionally, the key ecosystem function that bees perform (i.e., pollination) is influenced by the season-specific pollination effectiveness (Rafferty and Ives 2012) and temporal complementarity (Blüthgen and Klein 2011, Simanonok and Burkle 2014) of individual bee species. An explicit consideration of temporal diversity patterns is thus necessary to assess how anthropogenic disturbance affects bee assemblage structure and to identify potential consequences for ecosystem function.

Here, we explicitly examined the seasonal dynamics of our focal bee assemblages by simultaneously evaluating their temporal gamma, alpha, and beta diversity. Our use of linear mixed-effects models and analyses of multivariate dispersion (Anderson et al. 2006) distinguishes our study from previous work on temporal patterns in pollinator diversity, the majority of which has focused on quantifying the relative contributions of spatial versus temporal variation in structuring pollinator assemblages (Tylianakis et al. 2005, 2006, Schüepp et al. 2012, Kehinde and Samways 2014, Rollin et al. 2015). Our

approach enabled us to address the following research questions: (1) does habitat fragmentation affect all three components of bee temporal diversity similarly? And (2) how do the effects of habitat fragmentation vary with time? Addressing these research questions allowed us to scrutinize the impacts of habitat fragmentation with a temporal resolution that would be unachievable by pooling temporal samples within study sites.

MATERIALS AND METHODS

Study System: Between April and August of 2011 and 2012, we documented bee assemblages in the coastal sage scrub (CSS) ecosystems of San Diego County, California, USA, a global hotspot of bee biodiversity with over 500 bee species documented in the surrounding areas (Moldenke and Neff 1974, Michener 1979). We established 1-ha study plots in CSS habitat situated in (1) large natural reserves (internal area >> 500 ha), and (2) well-preserved habitat fragments (internal area < 120 ha, see Table 1-S1) embedded within the residential, urban matrix. In 2011, we surveyed four study plots in reserves and four study plots in fragments. In 2012, we surveyed seven study plots in reserves and 11 study plots in fragments. Details regarding the location and treatment classification (reserves or fragments) of each plot are provided in the Table 1-S1. Many of our study plots are located in the same system of reserves and fragments included in earlier studies on the ecological effects of urbanization-induced habitat fragmentation (Suarez et al. 1998, Laakkonen et al. 2001, Crooks et al. 2004), including bees sampled incidentally in pitfall traps (Hung et al. 2015). Permission to conduct field research was obtained from the University of California, San Diego; the Otay-Sweetwater Unit and Tijuana River National Estuarine Research Reserve Unit of the US National Wildlife Refuge; the City

of San Diego Open Space Parks Division and Real Estate Division; the City of La Mesa Open Space Division; and the City of Chula Vista Open Space Division.

Data collection: We employed bowl trapping and aerial netting (Westphal et al. 2008) to sample bees at all study plots, on sunny days with light wind. Bowl traps consisted of plastic bowls 7 cm in diameter that were white (left unpainted) or painted fluorescent blue or fluorescent yellow and filled with ca. 60 ml of unscented detergent solution. During each survey, 30 bowl traps were placed at a study plot before 0900 h and collected after 1500 h. Traps were placed on level ground in an alternating sequence of colors, deployed in two roughly linear transects originating from the corners of each plot and forming an "X" formation near the plot's center. Traps were placed 5-10 m apart from one another and at least 1 m from the canopy of large shrubs to avoid being shaded. During aerial netting, one researcher walked throughout the study plot and examined blooming plants as well as presumed nesting substrates (bare ground and dead, woody plant material) for bees. Non-*Apis* bee species were collected regardless of whether they were on flowers, in flight, or in the vicinity of presumed nesting substrates. In 2011, surveys were performed ca. every 2-3 weeks at each study plot (n = 9 survey days per plot), during which time, 60-min bouts of netting were performed once between 0900 h and 1200 h and once between 1200 h and 1500 h (120 min total per plot per survey). In 2012, in order to accommodate a larger number of study plots, surveys were performed ca. every 3-5 weeks (n = 5 survey days per plot) and included only a single 60-min bout of netting at each plot during each survey. Although seven sites were sampled in both years (Table 1-S1), the level of sampling employed here seems unlikely to have altered bee assemblages during our study (Gezon et al. 2015).

All collected bees were individually mounted and identified to species or morphospecies within genus using taxonomic keys and the reference collections of the American Museum of Natural History, UC Riverside Entomology Research Museum, California Academy of Sciences, UC Berkeley Essig Museum of Entomology, and UC Davis Bohart Museum of Entomology. Additionally, we also categorized each bee species as a pollen generalist or a pollen specialist based on whether it is documented to exclusively collect pollen from a single plant family. Data used to classify bees as generalists or specialists come from literature accounts for the species (or species group) (Hurd 1979) and its subgenus (Michener 2007), as well as our own field observations.

Bee assemblages often reflect the richness, abundance, and temporal dynamics of their host plant assemblages (Michener 1979, Wray and Elle 2015). Thus, concurrently with the bee sampling, we documented the identities of insect-pollinated native plant species present in each plot in each year; in 2012 we also counted the number of blooming individuals of each plant species in each plot during each survey. We documented blooming plants by walking through pre-planned paths that allowed the observer's field of view to cover the entirety of the study plot, as in (Burkle and Knight 2012), because many key plant species in our system are patchily distributed and because the thick growth of large, woody shrubs prohibited the use of random linear transects at many of our plots.

Statistical analyses: We compared native bee assemblages in reserve versus fragment plots with respect to their temporal gamma, alpha, and beta diversity. We analyzed data from each year separately because of differences in sample size and sampling frequency. In order to avoid human biases associated with aerial netting (e.g.,

catch rate may be reduced at sites where the collector's mobility is hindered by dense vegetation), our analyses include only bee specimens collected by bowl traps; however, inclusion of netted specimens in our analyses yielded qualitatively similar results. For analyses requiring species-level identification, we excluded 78 bee individuals (0.8% of individuals) not identifiable beyond genus. We also repeated all analyses at the genus level to ensure that particularly species-rich genera did not disproportionately influence our findings; the results of these additional analyses did not alter our main conclusions. Lastly, we verified that reserve and fragment plots did not differ with respect to the composition and temporal dynamics of insect-pollinated native plant assemblages, and that the plot-level compositions of bee assemblages were not spatially autocorrelated (see Appendix 1-1).

All analyses were conducted in R version 3.3.1 (R Development Core Team 2015); packages *vegan* (Oksanen et al. 2016), *MASS* (Venables and Ripley 2002), *car* (Fox and Weisberg 2011), and *nlme* (Pinheiro et al. 2016) were used in visualizing and analyzing data.

Temporal gamma diversity: We define temporal gamma diversity as the diversity of bees at a single study plot, pooled across all temporal samples (see Stegen et al. 2013), with each sample representing the bee specimens collected at one study plot during a single day of data collection. We considered both species richness and assemblage evenness (Pielou's *J*). In addition, we examined the proportion of bee individuals represented by generalist species (hereafter referred to as "generalist proportion"), as generalist bees can exhibit higher tolerance to anthropogenic disturbance compared to their specialist counterparts (Cane et al. 2006, Biesmeijer 2006). Lastly, we

also examined the temporal gamma component of bee abundance. We used rarefaction (repeated for 1,000 iterations) in our analyses of species richness and assemblage evenness to account for among-plot variation in the number of bees sampled. We used the lowest plot-level bee abundance recorded each year (n = 378 for 2011, n = 115 for 2012) as the number of individuals to subsample in our rarefactions. Bee abundance was calculated as the total number of bee individuals collected at each plot averaged across the number of temporal samples. Assemblage evenness and generalist proportion were logit-transformed prior to analysis as recommended by (Warton and Hui 2011), and bee abundance was cube root-transformed to improve normality. We used Welch's two-sample *t*-tests to compare fragment and reserve plots for all dependent variables listed above.

Given the dependence of bee diversity on the diversity and assemblage composition of their host plant assemblages (Michener 1979), we also repeated each analysis with the temporal gamma richness of native plants as an added independent variable (i.e., multiple regressions with treatment and plant richness as main effects). We then compared the corrected Akaike Information Criterion (AIC) scores (Akaike 1974) of each pair of models with or without plant richness added. Compared to original models that did not include plant richness, models that included plant richness yielded qualitatively similar results in all cases but had poorer (i.e., more positive) or equivalent AIC scores; thus, we did not include plant richness in our final models.

Temporal alpha diversity: We define temporal alpha diversity as the diversity of bees collected in a single temporal sample (see Tylianakis et al. 2005). As in our analyses of temporal gamma diversity, we examined species richness, logit-transformed

assemblage evenness, logit-transformed generalist proportion, and cube root-transformed bee abundance. In our analyses of species richness and assemblage evenness, we rarefied each temporal sample to 20 bee individuals (repeated for 1,000 iterations) to allow for unbiased comparisons between treatments and across temporal samples. In analyses requiring rarefaction, we excluded one sample from the 2011 dataset and nine samples from the 2012 dataset (including one fragment plot in which three of its five samples had fewer than 20 bees). We chose to rarefy to 20 individuals in order to minimize the number of data points to exclude while retaining sufficient resolution in our data.

To examine how bee assemblages in reserves and fragments differ over the course of the study period, we constructed linear mixed-effects models. This approach allowed us to quantify the direction of seasonal trends and to detect treatment-by-sample interactions, neither of which is possible for the additive diversity partitioning approach (Lande 1996) used by most published studies that examined bee temporal alpha diversity (see Tylianakis et al. 2005, 2006, Schüepp et al. 2012, Kehinde and Samways 2014, Rollin et al. 2015). In each model, treatment (fragment vs. reserve), temporal sample (the Julian date on which sampling occurred), and their interaction were included as fixed effects, and study plot identity was included as a random effect to control for repeated sampling as in (Wray and Elle 2015). To account for possible non-linear relationships between dependent variables and Julian dates of temporal samples, we constructed second- and third-degree orthogonal polynomial models in addition to first-degree linear models for each dependent variable, and selected the model with the lowest corrected AIC score. When alternative models yielded equivalent AICc scores ($\Delta AICc < 2$), the model with the lowest degree was chosen. Lastly, as with our analyses of temporal

gamma diversity, we repeated all analyses with the temporal alpha richness of native plants as an added independent variable (i.e., linear mixed-effects models including the main effects of treatment, temporal sample, and plant temporal alpha richness, and the interaction effect of treatment and temporal sample). Models that included plant richness yielded poorer AIC scores in all cases; thus, we did not include plant richness in our final models.

Temporal beta diversity: We define temporal beta diversity as the multivariate dispersion (Anderson et al. 2006, 2011) of bee assemblages in distinct temporal samples from the same study plot. We chose this index because of its relative mathematical independence from measures of alpha and gamma diversity (i.e., it is not calculated from the difference or ratio between alpha and gamma diversity), as well as its capability to detect differences among assemblages in both species identity and relative abundance (Anderson et al. 2006). Accounting for abundance makes multivariate dispersion less sensitive to rare species, which often make up a large fraction of the total species richness in bee assemblages (e.g., Williams et al. 2001, Fortel et al. 2014) but may contribute little to the pollination services rendered to plants (Vázquez et al. 2005). For these reasons, multivariate dispersion is superior to the traditional approach of using multiplicative (Whittaker 1972) or additive partitioning (Lande 1996) for investigating bee temporal beta diversity (e.g., Tylianakis et al. 2005, 2006, Schüepp et al. 2012, Kehinde and Samways 2014, Rollin et al. 2015) with respect to characterizing individual-level bee assemblage composition, as well as temporal turnovers in ecosystem function.

To calculate multivariate dispersion, we performed a non-metric multidimensional scaling (NMDS) ordination based on a dissimilarity matrix of

abundance-weighted bee assemblages in all possible pairs of samples across all plots (dissimilarity was calculated using the Bray-Curtis index, see Anderson et al. 2006). From this ordination, we calculated the multidimensional centroid of the samples from each plot, and then computed the mean distance between each plot's centroid and its constituent samples. The resulting dispersion score for each plot thus measures the degree to which the species composition of each plot's bee assemblage turns over through time. Dispersion scores of reserve and fragment plots were then compared using Welch's two-sample *t*-tests. As with our analyses of temporal gamma and alpha diversity, we repeated all analyses with the temporal beta diversity of native plants as an added independent variable (i.e., multiple regression models with main effects of treatment and plot-level multivariate dispersion of plant assemblages). Models that included plant temporal beta diversity yielded poorer AICc scores in all cases; thus, we did not include plant temporal beta diversity in our final models.

RESULTS

In two years of sampling, we collected 12,036 bee specimens belonging to 235 species (185 described species and 50 additional morphospecies) in 54 genera and 6 families (Hung and Holway 2017). Bowl trapping yielded 9,421 specimens (82%) belonging to 168 species (71%), while aerial netting yielded 2,128 specimens (18%) belonging to 179 species (76%). We identified 11,376 specimens (94.5%) to described species, including 485 honey bee workers (*Apis mellifera* L.) and two specimens of non-native wild bees: one female *Megachile rotundata* (Fabricius) and one male *Hylaeus leptocephalus* (Morawitz). A total of 497 specimens (4.1%) was assigned to

morphospecies. Species-level identification for this latter set of bees was hindered by the lack of taxonomic revisions and comprehensive reference collections for many bee genera in this region, evidenced by the ongoing discovery of undescribed species (e.g., Rightmyer et al. 2014). Finally, 164 specimens (1.4%) were not identified to species (or morphospecies) as they were rendered unidentifiable beyond genus (or subgenus) due to weathering or other damage, or were male morphospecies that could not be confidently associated with females.

Temporal gamma diversity: In both years, fragment plots harbored bee assemblages with significantly lower rarefied species richness (Fig. 1-2A; on average 36% lower) as well as lower rarefied assemblage evenness (Fig. 1-2B; on average 18% lower). Fragments also harbored bee assemblages that had higher generalist proportions compared to those in reserves (Fig. 1-2C; on average 7% higher). However, reserves and fragments did not differ in bee abundance in either year (Fig. 1-2D).

Temporal alpha diversity: Linear mixed-effects models revealed that fragment plots supported bee assemblages with significantly lower rarefied species richness (Figs. 1-3A and 1-3E; $F_{1,6} = 12.89$, $P = 0.012$ in 2011 and $F_{1,15} = 13.25$, $P = 0.002$ in 2012), with richness decreasing as the Julian date of the sample increased. The relationship between richness and Julian date was linear in 2011 ($F_{1,61} = 30.77$, $P < 0.0001$) and parabolic in 2012 ($F_{1,58} = 35.99$, $P < 0.0001$ for Julian date and $F_{1,58} = 16.07$, $P = 0.0002$ for Julian date^2). Similarly, assemblage evenness was lower in fragments, at least in 2011 (Figs. 1-3B and 1-3F; $F_{1,6} = 6.06$, $P = 0.049$ in 2011 and $F_{1,15} = 3.97$, $P = 0.065$ in 2012). While assemblage evenness decreased throughout the study period in 2011 ($F_{1,61} = 5.49$, $P = 0.022$), there was no such seasonal effect in 2012 ($F_{1,60} = 2.09$, $P = 0.15$). Generalist

proportion was higher in fragments than in reserves in both years (Figs. 1-3C and 1-3G; $F_{1,6}$ = 7.80, P = 0.032 in 2011 and $F_{1,16}$ = 8.25, P = 0.011 in 2012), and increased throughout the study period ($F_{1,62}$ = 35.37, P < 0.0001 in 2011 and $F_{1,68}$ = 64.75, P < 0.0001 in 2012).

There was a significant treatment-by-sample interaction for bee abundance in 2011 (Fig. 1-3D; $F_{1,58}$ = 5.25, P = 0.026 for the interaction involving Julian date; $F_{1,58}$ = 0.10, P = 0.75 for the interaction involving Julian date^2; and $F_{1,58}$ = 1.48, P = 0.22 for the interaction involving Julian date^3) as well as in 2012 (Fig. 1-3H; $F_{1,68}$ = 4.29, P = 0.042), wherein bee abundance was generally higher in reserves earlier in the study period and higher in fragments later in the study period. In 2011, abundance varied roughly sinusoidally as the Julian date of the sample increased ($F_{1,58}$ = 13.88, P = 0.0004 for Julian date; $F_{1,58}$ = 0.27, P = 0.61 for Julian date^2; and $F_{1,58}$ = 14.30, P = 0.0004 for Julian date^3); however, in 2012, abundance did not vary with temporal sample ($F_{1,68}$ = 1.80, P = 0.18). In the overall model, bee abundance did not differ between reserves and fragments ($F_{1,6}$ = 0.56, P = 0.48 in 2011 and $F_{1,16}$ = 0.08, P = 0.78 in 2012).

Temporal beta diversity: The temporal beta diversity of bee assemblages was significantly lower in fragments than in reserves in 2012 (Fig. 1-4; ca. 26% lower). Results from 2011 exhibited similar trends (diversity was ca. 19% lower in fragments compared to reserves); however, the comparison in this year did not quite attain statistical significance at the $\alpha = 0.05$ level (Fig. 1-4).

DISCUSSION

Across our two years of sampling, we found consistent differences in bee assemblages occurring in reserves and fragments, despite the known tendency for bee faunas to exhibit considerable inter-annual variation at a given locality (Williams et al. 2001). Compared to reserves, fragments harbored bee assemblages that were less diverse with respect to all three components of temporal diversity (Figs. 1-2, 1-3, and 1-4). While all metrics of bee diversity and abundance varied with time, differences in bee diversity between reserves and fragments were remarkably constant through time (Fig. 1-3). Individually scrutinizing the three components of temporal diversity allowed for a high-resolution characterization of the temporal structure (Fig. 1-1) of bee assemblages in intact and fragmented habitats; these analyses also yielded further insights into the potential consequences of bee diversity loss for ecosystem function in fragmented habitats in our system.

Reduced species richness is one of the most commonly reported effects of habitat fragmentation on bee assemblages (Winfree et al. 2009). Though our reserve and fragment plots did not differ systematically with respect to the composition of floral resources (Appendix 1-1), it is possible that decreased availability of nest sites within foraging distance of key host plants (Westrich 1996, Zurbuchen et al. 2010) or increased vulnerability to demographic stochasticity due to isolation (Cane 2001) or small population size may have contributed to reduced bee species richness in fragments. Analyses of the temporal gamma and temporal alpha components of bee species richness yielded qualitatively similar results; however, the impact on each of the two temporal diversity components may have distinct implications for the conservation of bees and

ecosystem function. The temporal gamma component of bee richness provides information on the habitat conditions and locations that support the greatest total number of bee species or species of particular conservation concern; as such, it is the most useful metric for developing conservation strategies aimed at bees. On the other hand, the pollination effectiveness of a particular bee species for a particular plant species may depend upon the timing during which the interaction between bees and plants takes place (Rafferty and Ives 2012) or upon the bee species' functional complementarity with other, temporally co-occurring pollinator species (Blüthgen and Klein 2011). Detecting potential impacts of climate change on the phenological matching between bee species and the plants they pollinate (Benadi et al. 2014, Rafferty et al. 2015) also requires examining the composition of bee assemblages at discrete points in time. Thus, in the face of a changing climate, effective strategies aimed at conserving bees and the ecosystem function they perform should account for both the temporal alpha and gamma components of bee richness.

As with patterns of bee species richness, patterns in the temporal gamma and alpha components of bee assemblage evenness are in qualitative agreement with each other. Assemblage evenness is an important driver of ecosystem function (reviewed in Hillebrand et al. 2008), including pollination (Balvanera et al. 2005), but remains an under-appreciated aspect of pollinator assemblage dynamics (Marini et al. 2014). Reductions in the temporal alpha component of bee assemblage evenness in fragments may result in decreased frequencies of interspecific encounters among bee species; such encounters have been shown to enhance pollination efficiency via altering bee foraging behavior (Greenleaf and Kremen 2006). On the other hand, reductions in the temporal

gamma component of bee assemblage evenness may result in a stronger reliance by plant assemblages on a small subset of numerically dominant bee species, and consequently, reduced stability of pollination services (Balvanera et al. 2005).

In contrast to patterns of bee species richness and assemblage evenness, overall bee abundance did not differ between reserves and fragments. This pattern was caused by reserves having higher bee abundance in spring (April through early June) and fragments having higher bee abundance in summer (late June through August; Figs. 1-3D and 1-3H). This treatment-by-sample interaction appears to be driven by the higher relative abundance of generalist bees in fragments (Figs. 1-2C, 1-3C, and 1-3G); many generalist species in our system (e.g., many primitively eusocial halictine species) reach peak abundance between late June and August. Generalist bees may be more tolerant of habitat fragmentation compared to specialists (e.g., Cane et al. 2006) and have been hypothesized to replace the ecosystem function formerly performed by extirpated specialists (Memmott et al. 2004). However, even though generalists in our study numerically compensated for absent specialists when considering the temporal gamma component of bee abundance (Fig. 1-2D), reduced bee abundance in fragments early in our study period (April through early June) may threaten the pollination of spring-blooming plant species.

Temporal beta diversity represents another under-appreciated metric in ecology (Korhonen et al. 2010), and reports on the effects of anthropogenic disturbance on intra-annual turnover of biological assemblages remain rare (e.g., Tylianakis et al. 2005, Lauber et al. 2013, Uchida and Ushimaru 2015). In our system, decreased temporal beta diversity in fragments may explain how modest reductions in the temporal alpha

component of species richness and assemblage evenness in fragments (Fig. 1-3) translate into more pronounced reductions in the temporal gamma component (Fig. 1-2). More broadly, decreasing seasonal turnover in an assemblage may result in increasing temporal niche overlap among its constituent species (Wilsey et al. 2011), which may in turn decrease the number of distinct temporal niches created by the assemblage. Decreases in the seasonal turnover of bee assemblages may be especially consequential in cases where bee species tend to interact with a set of preferred host plants throughout their activity season even when new plant species begin to bloom as time progresses (e.g. Simanonok and Burkle 2014). If temporal host-switching is likewise rare in our system, reduced bee assemblage turnover in fragments may jeopardize the reproduction of certain plant species that occupy specific temporal niches with respect to pollination (Blüthgen and Klein 2011). Examining the temporal beta diversity of bee assemblages thus appears crucial for understanding mechanisms underlying the impact of anthropogenic disturbance on pollination services.

CONCLUSIONS

Our synthesis of the three components of bee temporal diversity revealed that CSS fragments in our system support bee assemblages that (1) have lower species richness and evenness but higher numerical dominance by generalists at any given point in the bee activity season, (2) reach peak abundance later in the bee activity season (late June through August), and (3) exhibit less temporal turnover. Correspondingly, these patterns suggest that plants occurring in CSS fragments may suffer from decreases in (1) the functional complementarity of bee taxa that simultaneously co-occur, (2) floral

visitation by bees early in the bee activity season (April through early June), and (3) the number of available temporal niches with respect to pollination. Our research demonstrates the potential importance of quantifying distinct components of temporal diversity when characterizing the impacts of anthropogenic disturbance on seasonal organisms, as well as when predicting how such impacts may influence ecosystem function. As human activity and climate change continue to alter Earth's ecosystems, it will be increasingly important to document how anthropogenic disturbance impacts assemblage structure and ecosystem functions associated with distinct components of temporal diversity in organisms that exhibit inherent seasonal dynamics.

ACKNOWLEDGEMENTS

We gratefully acknowledge the taxonomic assistance of J. Gibbs, T. Griswold, M. Orr, Z. Portman, M. Rightmyer, S. Sipes, R. Thorp, K. Wright, and D. Yanega, field and laboratory assistance of S. Fung, W. Wen, R. Graham, C. Johnson, J. May, and S. Plesim, and constructive comments on our analyses and manuscript by K. Boersma, D. Bolger, E. Cleland, R. Deutsch, R. Irwin, J. Kohn, R. Lande, L. Levin, J. Minster, J. Nieh, S. Rifkin, C. Sidhu, A. Suarez, C. Symons, A. Traveset, and E. Wolkovich. This work was performed in scrub habitats managed by the University of California Natural Reserve System (Scripps Coastal Reserve and Elliott Chaparral Reserve), Open Space Parks in San Diego County (cities of Chula Vista, La Mesa, and San Diego), the Otay-Sweetwater Unit of the San Diego National Wildlife Refuge, the and the Tijuana River National Estuarine Research Reserve. This work was funded by the UC San Diego Academic Senate Bridge Fund Grant BIO088B, the NSF Doctoral Dissertation Improvement Grant

DEB-1501566, the National Geographic Society Young Explorer Grant 9014-11, the Mildred E. Mathias Graduate Student Research Grant from the University of California Reserve System, the Jeanne Marie Messier Memorial Endowed Fund, and Sigma Xi Grant in Aid of Research G20111015158245.

Chapter 1, in full, has been submitted for publication of the material as it may appear in *PLOS ONE*, 2017, Hung, Keng-Lou J.; Ascher, John S.; Holway, David A., Public Library of Science, 2016. The dissertation author was the primary investigator and author of this manuscript.

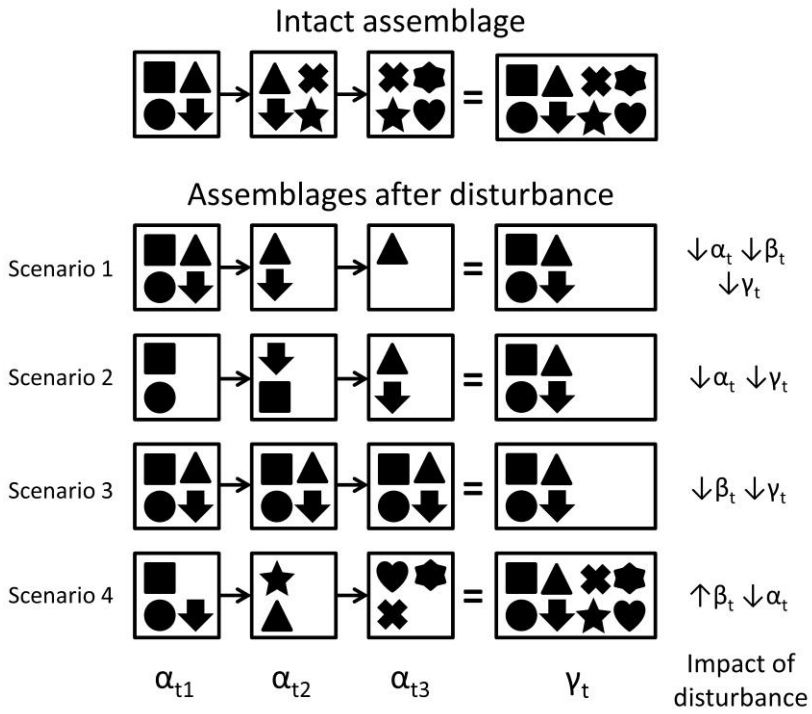

Figure 1-1. Hypothetical scenarios of disturbance impacting temporal gamma, alpha, and beta diversity of an assemblage. Each symbol represents a distinct taxon; α_t represents taxon richness at discrete time points, β_t represents the turnover of taxa between time points, and γ_t represents taxon richness summed across time points.

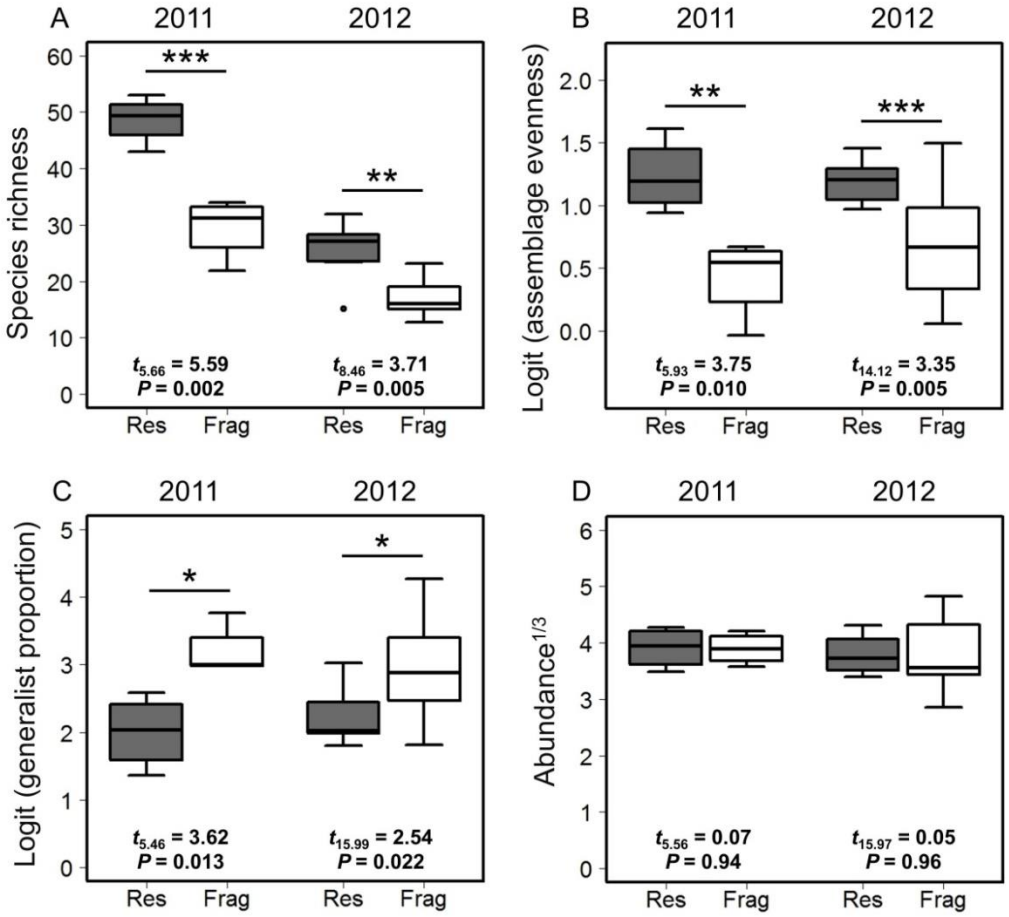

Figure 1-2. Temporal gamma diversity of native bees in reserve (gray boxes) and fragment plots (white boxes). Plots show (A) rarefied species richness, (B) logit-transformed rarefied assemblage evenness (Pielou's *J*), (C) logit-transformed proportion of individuals belonging to generalist species, and (D) cube root-transformed average number of bees collected per temporal sample.

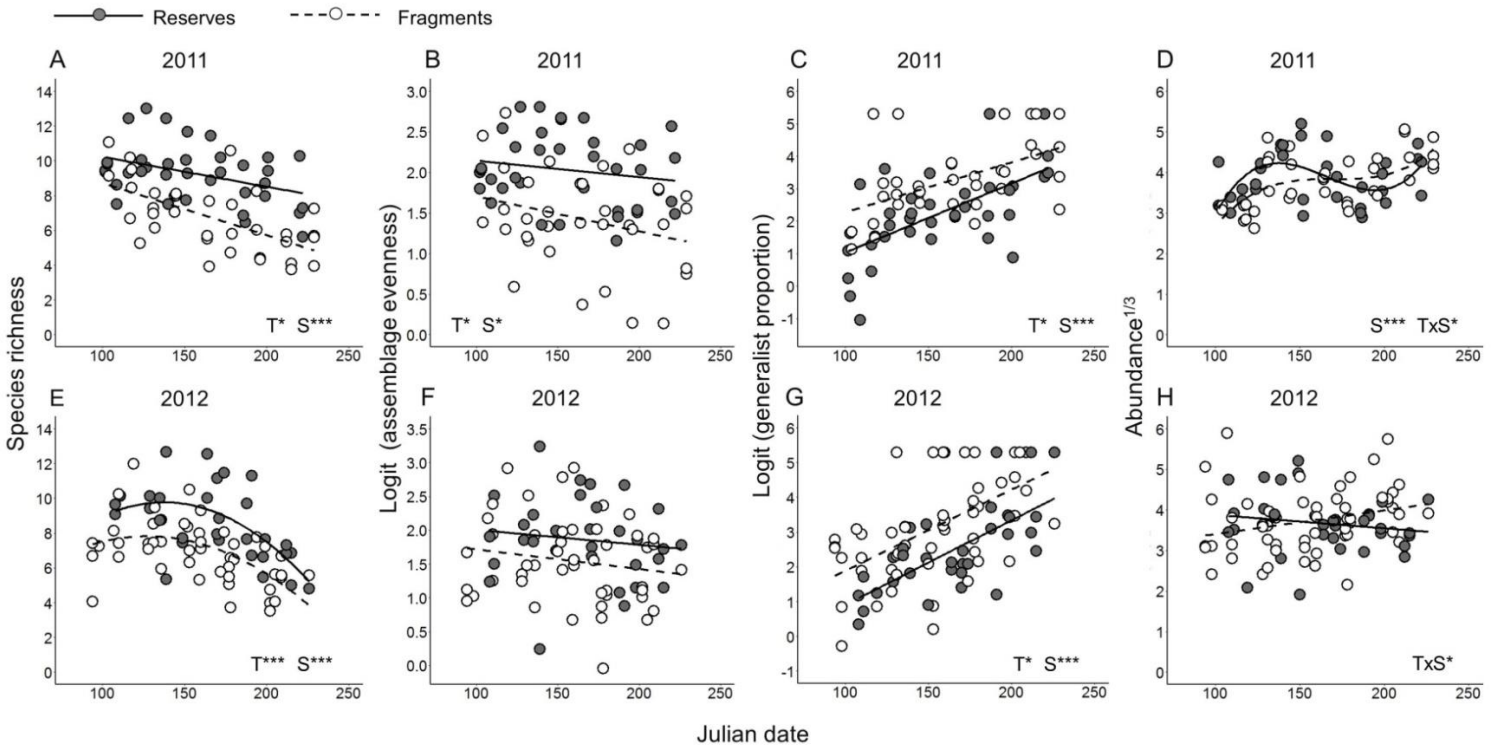

Figure 1-3. Temporal alpha diversity of native bees in reserve and fragment plots in 2011 (A-D) and 2012 (E-H). Plots show (A,E) rarefied species richness, (B,F) logit-transformed rarefied assemblage evenness (Pielou's *J*), (C,G) logit-transformed proportions of individuals belonging to generalist species, and (D,H) cube root-transformed number of bees collected per temporal sample. All plots show regression lines to visualize data trends. Significant main effects and interactions from linear mixed-effects models are indicated on each graph: T = treatment, S = temporal sample. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

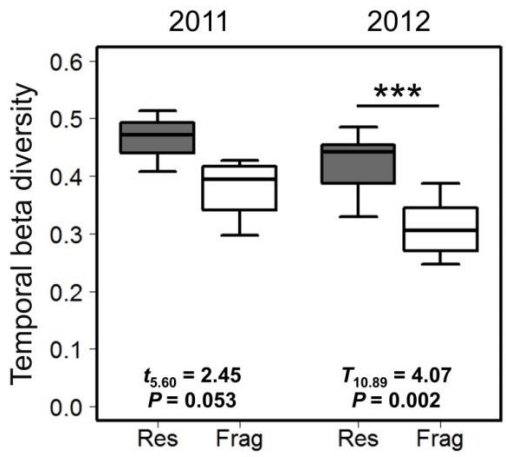

Figure 1-4. Temporal beta diversity of native bees in reserve (gray boxes) and fragment plots (white boxes). Beta diversity was calculated as the multivariate dispersion of abundance-weighted bee assemblages in distinct temporal samples within each study plot.

Appendix 1-1: Description and explanation of study sites.

We chose study plots in sites that contained a diversity of native shrubs, dominated by combinations of *Acmispon glaber* (Vogel) Brouillet, *Artemisia californica* Less., *Bahiopsis laciniata* (A. Gray) E. E. Schilling & Panero, *Eriogonum fasciculatum* Bentham, *Malosma laurina* (Nutt.) Abrams, *Rhus integrifolia* (Nutt.) Brewer & S. Watson, and *Salvia mellifera* E. Greene. To the extent possible, we chose sites with minimal invasion by exotic forbs such as *Brassica nigra* (L.) Koch and *Erodium* spp., and exotic grasses such as *Avena* spp. and *Bromus* spp.

Reserve plots were chosen within four distinct reserves: Elliott Chaparral Reserve of the University of California Reserves System (plots ECR1 and ECR2), Mission Trails Regional Park (plots MTE2 and MTI2), the Otay-Sweetwater unit of the San Diego National Wildlife Refuge (plots SWEA and SWI2), and the Tijuana River National Estuarine Research Reserve (plot TRR1). Fragment plots were chosen in the vicinity of reserve plots (maximum distance between fragment and reserve plots < 15 km) and consisted of well-preserved scrub habitat surrounded by urban, residential infrastructure such as roads (two lanes minimum), buildings, and paved lots (and in one instance, a sandy beach). Details on each study plot are given in Table 1-S1. Permission to perform research was obtained from the University of California Natural Reserves System, the National Wildlife Refuge, the City of San Diego, the City of Chula Vista, and the City of La Mesa. Plots are separated from their nearest neighbors by a minimum of 1.5 km, except for two pairs of plots which are separated from their nearest neighbors by ca. 500 m. Despite the fact that six of our reserve plots represented relatively closely-situated pairs of plots in three natural reserves, we opted to treat them as independent replicates for two reasons. First, Mantel Tests reveal no spatial autocorrelation with respect to bee assemblage composition as calculated by the Bray-Curtis dissimilarity index ($r = 0.073$, $P > 0.05$ in 2011; $r = 0.15$, $P > 0.05$ in 2012). Second, excluding one plot from each pair of closely-situated plots from our analyses did not change our main conclusions.

To verify that our reserve and fragment study plots contained plant assemblages similarly representative of intact coastal sage scrub, we compared reserve and fragment plots with respect to insect-pollinated native plant species richness and the abundance of perennial, insect-pollinated shrubs. We also compared the composition of native plant assemblages in reserve and fragment plots using permutational multivariate ANOVAs (PERMANOVAs). Lastly, we also examined the multivariate dispersion of temporal samples of plants as a metric of the temporal turnover of plant assemblages. Our estimates of the presence-absence (in 2011) or number of individuals (2012) of each plant species in bloom did not allow for direct comparisons of the abundance and evenness of floral resources across sites and across plant species because of variation in plant sizes across sites and across species. Thus, we performed PERMANOVAs and calculated multivariate dispersions of plant assemblages based on presence-absence data rather than count data (for calculations of multivariate dispersion, plant species that were blooming during a survey round was scored as “present,” all other plant species were scored as “absent”). Compared to analyses using abundance-weighted data, analyses using presence-absence data yield results that are lower in resolution but nevertheless qualitatively similar.

Native plant species richness did not differ between reserves and fragments in 2011 (two-sample *t*-test $t_{3.66} = 0.84$, $P > 0.05$) or 2012 ($t_{15.36} = 0.92$, $P > 0.05$). Similarly, shrub abundance did not differ between reserves and fragments in 2012 ($t_{13.97} = 0.67$, $P > 0.05$); shrub abundance was not recorded with sufficient resolution in 2011 to allow comparison between reserves and fragments. Reserves and fragments also did not differ with respect to native plant assemblage composition in 2011 (PERMANOVA $F_{1,6} = 0.88$, $P > 0.05$) or 2012 ($F_{1,16} = 1.25$, $P > 0.05$). Lastly, plant temporal beta diversity did not differ between fragments and reserves in 2011 (two-sample t-*t*est $t_{4.10} = 0.66$, $P > 0.05$) or 2012 ($t_{15.01} = 0.68$, $P > 0.05$).

Table 1-S1. List of utilized study plots in coastal sage scrub reserves and habitat fragments.

Plot	Yr. sampled	Frag / Res	Latitude	Longitude	Internal area (ha)
MTLB1	2011	Fragment	32.800	-117.137	116.69
CFS1	2012	Fragment	32.814	-117.237	36.44
MTS2	2012	Fragment	32.856	-117.188	12.89
MTS3	2012	Fragment	32.787	-117.141	31.96
MTS6	2012	Fragment	32.722	-117.119	52.79
MTS7	2012	Fragment	32.740	-117.086	12.01
SCR	2012	Fragment	32.875	-117.248	36.38
SWS10	2012	Fragment	32.786	-116.989	6.23
TRS1	2012	Fragment	32.632	-117.033	9.19
MTS1A	2011-12	Fragment	32.792	-117.061	2.72
SWS1	2011-12	Fragment	32.750	-117.032	46.55
SWS3	2011-12	Fragment	32.720	-117.078	28.06
ECR1	2012	Reserve	32.892	-117.092	> 500
ECR2	2012	Reserve	32.889	-117.096	> 500
TRR1	2012	Reserve	32.565	-117.126	> 500
MTE2	2011-12	Reserve	32.834	-117.078	> 500
MTI2	2011-12	Reserve	32.842	-117.065	> 500
SWEA	2011-12	Reserve	32.732	-116.956	> 500
SWI2	2011-12	Reserve	32.734	-116.950	> 500

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CHAPTER 2: Parallel Loss of Taxonomic and Functional Diversity in Native Bee Assemblages Inhabiting Fragments of Well-Preserved Scrub Habitat in Southern California

ABSTRACT

Human modification of natural habitats often generates novel selection forces, or ecological filters, that restructure biological communities. Understanding the extent to which novel ecological filters drives biodiversity loss is important for predicting long-term consequences of habitat alterations for ecosystem function and conservation. We assessed the strength of ecological filters in restructuring assemblages of native bees inhabiting a fragmented scrub ecosystem by collecting native bees in large natural reserves and well-preserved fragments of scrub habitat embedded in an urban landscape. We compared reserve and fragment sites with respect to both taxonomic and functional diversity and composition of bee assemblages. We also investigated the degree to which bee assemblages in fragments were more numerically dominated by geographically widespread bee species. We found that bee assemblages in fragments exhibited reduced taxonomic and functional diversity, as well as distinct taxonomic and functional composition, relative to reserves. Fragments also exhibited an increase in the relative abundance of bees with larger geographical ranges. However, despite the detection of indicator species and functional groups that are associated with reserve sites, fragments did not exhibit reduced spatial beta diversity relative to reserves, and overall patterns of functional diversity loss in fragments are consistent with random species loss predicted

by null models. We demonstrated that ecological filters reduced bee diversity and shifted bee community composition even in well-preserved habitat fragments, underscoring the importance of preserving large areas of natural habitat for conserving both the taxonomic and functional diversity of bee assemblages. However, fragments each retained distinctive bee faunas such that, in aggregate, fragments may preserve a substantial portion of the local bee species pool despite the presence of ecological filters.

INTRODUCTION

The alteration of natural habitats by human activities is the leading cause of biodiversity loss worldwide (Vitousek et al. 1997). Novel selection forces in altered habitats often create ecological filters (Mayfield et al. 2005)—environmental or biotic processes that determine which species can establish or persist. The strength of ecological filters depends on the form of disturbance, the natural history of organisms in question, and the strength of other forces shaping community assembly such as dispersal, competition, and natural disturbance regimes (e.g., Orrock and Watling 2010, Myers and Harms 2011). Understanding the extent to which ecological filters shape community assembly represents a central goal of community ecology (Chase and Myers 2011), and can be used to predict the long-term implications of habitat modifications (Myers and Harms 2011, Püttker et al. 2015).

Assessing the strength of ecological filters is especially important when evaluating the long-term ecological consequences of habitat fragmentation, one of the leading causes of ecosystem change and biodiversity loss worldwide (Fahrig 2003). Many studies have found strong evidence that ecological filtering drives diversity loss in

habitat fragments, both at the local scale and landscape scale (Tabarelli et al. 2012, Bregman et al. 2015, Farneda et al. 2015). On the other hand, community assembly in fragmented landscapes may also be shaped by stochastic colonization and extinction events (Gilbert et al. 2006, Orrock and Watling 2010) typical of island biogeography (MacArthur and Wilson 1967), or by underlying heterogeneity among habitat patches (Tscharntke et al. 2002, Sfair et al. 2016). While habitat fragmentation could reduce local species richness through different combinations of the abovementioned processes, effective conservation practices will need to account for the degree to which ecological filtering drives species loss (Püttker et al. 2015, Ulrich et al. 2016).

One powerful approach to assess the strength of ecological filters in fragmented habitats is to examine functional diversity, since ecological filters, by definition, act on functional traits rather than species (Cadotte et al. 2011). Functional diversity is related to taxonomic diversity in complex ways (Mayfield et al. 2010), and the relationship between the two metrics can provide insight into the mechanisms that drive biodiversity loss in fragmented habitats. For instance, when habitat fragmentation results in strong ecological filtering, functional diversity may decline even if species richness and abundance remains little altered, as may be the case when taxa that thrive in fragmented habitats replace those that are extirpated (Mayfield et al. 2010). On the other hand, if species loss in habitat fragments mainly results from stochastic extinction events associated with small population size and isolation, functional diversity may be relatively unaffected by the loss of taxonomic diversity, especially in systems with sufficient functional redundancy among species (Fonseca and Ganade 2001).

Here, we evaluate the contribution of ecological filters to diversity loss by taking advantage of an extensive survey of native bees (Hymenoptera: Anthophila) in a species-rich ecosystem where we have documented profound reductions in bee species richness associated with urbanization-induced habitat fragmentation (see Chapter 1 of this dissertation). Bees are ecologically important pollinators (Kearns and Inouye 1997) known to exhibit non-random species loss in fragmented habitats, where specialist species appear particularly vulnerable (Cane et al. 2006). Ecological filtering of bees may occur in habitat fragments when fragments experience reductions in the diversity or abundance of plant species (Soulé et al. 1992) that serve as food resource for bees, exhibit altered abiotic conditions due to influences from the surrounding matrix (Driscoll et al. 2013), or fail to contain the correct spatiotemporal configuration of food and nesting resources (Westrich 1996). Habitat fragmentation may also reduce bee diversity via processes not related to ecological filtering; for instance, when the isolation of habitats disrupts dispersal processes crucial in buffering bee populations from year-to-year variation in the local and temporal distribution of floral resources (Williams et al. 2001).

We assess the strength of ecological filters by addressing four questions. First, to what extent does fragmentation impact bee functional diversity? Loss of functional diversity more severe than that predicted by a null model of random species loss would lend support for the importance of ecological filters (Mayfield et al. 2010). Second, do bee assemblages in fragments exhibit distinct taxonomic or functional compositions compared to those in reserves, as would be expected when ecological filtering causes the assembly of novel communities (Boersma et al. 2016)? Third, do bee assemblages in fragments exhibit lower taxonomic or functional beta diversity among plots, as would be

expected when ecological filters select for or remove common sets of functional traits in altered habitats (Lôbo et al. 2011, Karp et al. 2012, Gámez-Virués et al. 2015, Liu et al. 2016)? Lastly, are bee assemblages in fragments composed of taxa with larger range sizes relative to those in reserves? Given that range size tends to be positively related to niche breadth (Brown 1984, Slatyer et al. 2013), a shift to more cosmopolitan species in fragments is expected if ecological filtering precludes the persistence or (re)colonization of species more specialized to the local ecosystem (McKinney and Lockwood 1999). Answering these questions will yield insights into the mechanisms that drive bee species loss in our study system as well as provide information on the potential conservation value of scrub habitat fragments (Tscharntke et al. 2002).

METHODS

Study system: Field data were collected between April and August of 2011 and 2012 in coastal sage scrub (CSS) habitat in San Diego County, California, USA, and are detailed in Chapter 1 of this dissertation. We surveyed one-hectare study plots belonging to two categories: (1) large natural reserves (internal area >> 500 ha), and (2) habitat fragments (internal area < 120 ha) surrounded by urban development. This is the same system of reserves and fragments previously used to study the ecological effects of urbanization-induced habitat fragmentation (Suarez et al. 1998, Crooks et al. 2004, Bolger et al. 2008). In 2011, we surveyed eight study plots (n = 4 for each category) ca. every 2-4 weeks; in 2012, we surveyed 17 study plots (n = 6 reserve plots; n = 11 fragment plots) ca. every 3-5 weeks. During each survey at each study plot, the first author deployed 30 bowl traps (10 each of fluorescent blue, fluorescent yellow, and white)

between ca. 0900 h and 1500 h, and collected free-flying wild bees via aerial netting (120 min per survey day in 2011; 60 min per survey day in 2012). Concurrently, we also documented the identities of native, insect-pollinated plant species in bloom at each study plot by walking through pre-planned paths that allowed an observer to visually survey the entire study plot (as in Burkle & Knight, 2012). All collected bees were individually mounted and identified to species or morphospecies within genus, hereafter referred to collectively as "species" (see Chapter 1 of this dissertation). This sampling effort resulted in a dataset of 11,037 native bees belonging to 216 species in 52 genera and 6 families, after the exclusion of bee specimens not identifiable beyond genus (i.e., bees damaged by weathering and male morphospecies not matched to females, accounting for < 2% of the dataset).

The fragment sites exhibited marked reductions in bee species richness and assemblage evenness relative to reserve sites (see Chapter 1 of this dissertation, Fig. 1-2), making this an excellent system in which to examine the extent to which ecological filtering causes the restructuring of assemblages. Our dataset also possesses a number of other desirable properties. First, since the original intent of data collection was to examine the effect of habitat fragmentation in isolation from other effects of urbanization-induced landscape change (e.g., habitat degradation, proliferation of non-native organisms), we selected study plots representative of intact CSS flora to the extent feasible. Accordingly, reserves and fragments did not differ with respect to the plot-level species richness or composition of native insect-pollinated plants (See Chapter 1 of this dissertation, Appendix 1-1). Second, study plots exhibited no spatial autocorrelation with respect to the species composition of native bee assemblages, minimizing the potential

for patterns in the spatial arrangement of study plots to drive patterns of bee taxonomic or functional distribution. Lastly, reserves and fragments did not differ with respect to overall bee abundance (see Chapter 1 of this dissertation, Fig. 1-2); minimizing the potential for sampling effects to contribute to any differences that we detect between reserve and fragment plots with respect to bee taxonomic or functional composition.

Functional trait assignment and analyses of functional diversity: Every identified bee species was assigned a category with respect to each of the following natural history traits: lecty (i.e., pollen diet breadth), nest location, nest building behavior, sociality, body size, and flight season. Table 2-1 lists each trait, how it is analyzed in functional diversity models (e.g., as a continuous, binary, or categorical variable), and its method of assignment; Table 2-S1 lists the bee species and their associated traits. With the exception of body size and flight season, all traits were assigned to individual species using literature syntheses for the species or species group (Hurd 1979) and subgenus (Michener 2007), as well as revisionary publications on lower taxa and our own field observations. Due to the lack of data for many species in our region, we also relied on phylogenetic inference when such data are available and appropriate: e.g., all *Lasioglossum* (*Dialictus*) species were scored as polylectic, ground-nesting, actively constructing nests, and eusocial (Michener 2007). Cleptoparasitic bees (37 species, n = 177 specimens) are included in all analyses, although excluding them does not qualitatively alter our results. Cleptoparasites are classified to a unique lecty category (given that they are limited to only the pollen resources collected by their host bees at any given locality), the same nest location as their presumed hosts, and always as nest renters. Although some species of *Sphecodes* in our system may be social parasites of eusocial

Halictini species (Michener 2007), we classified all cleptoparasites as solitary because they do not exhibit reproductive division of labor (Michener 2007, Table 2-1).

Two traits, body size and flight season, were not assigned based on published data. To estimate body size, we measured the intertegular lengths (see Cane 1987) of three haphazardly selected females of each solitary species or four haphazardly selected females of each eusocial species, when possible. For species for which no females were collected, we measured the intertegular lengths of males. To assign flight season, we assembled an individual-level database of collection dates from our own field data and the database of the University of California, Riverside Entomology Museum. Since climatic conditions may drive intraspecific variation in the timing and duration of bee flight seasons, we including only specimen records from south of 36.00° latitude, within 80 km of the Pacific coast, and below an altitude of 1000 m. From this database, we performed 1,000 random subsamples of 30 specimens per species and calculated the tenth and ninetieth percentile collection dates (see also Forrest et al. 2015). We then scored each bee species with respect to whether or not they are active in the early, middle, and late part of our study period using these percentiles. Rather than assigning quantitative measures of flight season duration and median flight date (Forrest et al. 2015), our approach of assigning flight season as presence-absence in coarser-grained season categories (see also Tonietto et al. 2016) minimizes biases inherently present in most databases, such as non-uniform distribution of sampling dates and non-random captures of different taxa by different collectors. This approach also minimizes the impact of the sampling effect, in which rarer taxa in the database are likely to be recorded as having

shorter flight seasons (e.g., in extreme examples, singletons would be recorded as being active for a single day).

For our metric of functional diversity, we chose functional dispersion ("FDis", see Laliberté and Legendre 2010), a widely used metric (Audino et al. 2014, Gagic et al. 2015, Boersma et al. 2016) that can provide insight into how native bee functional diversity responds to anthropogenic alterations of natural habitat (Forrest et al. 2015, Tonietto et al. 2016). FDis is calculated as the mean distance of each species from its site-level, multivariate centroid (Anderson et al. 2006) of functional traits (Laliberté and Legendre 2010). Thus, FDis is mathematically independent of species richness and can take into account differences in the relative abundances of functional trait combinations (Laliberté and Legendre 2010). These attributes make FDis relatively insensitive to rare species and functionally equivalent species; thus FDis particularly well-suited for our dataset, in which we have detected strong differences between reserves and fragments in both species richness and assemblage evenness (see Chapter 1 of this dissertation, Fig. 1-2)

Statistical analyses: Except where noted otherwise, we analyzed data from 2011 and 2012 separately because of differences in sampling location and frequency. In order to avoid biases associated with variation in aerial netting efficacy in different terrains, bees collected by aerial netting were excluded from our main analyses; however, inclusion of these netted specimens in our analyses yielded qualitatively similar results (Table 2-S2). We also repeated all analyses at the genus level using genus-level mean or modal averages for functional traits to ensure that particularly species-rich genera did not disproportionately influence our findings. The results of these genus-level analyses also

did not alter our main conclusions (Table 2-S2). All analyses were conducted in R version 3.3.1 (R Development Core Team 2015).

Taxonomic and functional alpha diversity: To assess plot-level alpha diversity, we calculated Shannon-Weiner diversity *H* (using R package *vegan* (Oksanen et al. 2016)) and FDis (using R package *FD* (Laliberté and Legendre 2010)), where each diversity metric was weighted by relative abundances of each species. To account for variation in the number of bees sampled per plot, we calculated both diversity metrics after rarefying our data (repeated for 1,000 iterations) to the lowest plot-level bee abundance recorded each year (n = 378 for 2011, n = 115 for 2012). Diversity metrics in reserves and fragments were compared with two-sample *t*-tests. Additionally, we constructed a linear mixed-effects model (using R packages *lme4* (Bates et al. 2015), *lmerTest* (Kuznetsova et al. 2016), and *MuMIn* (Barton 2016)) to examine the relationship between taxonomic and functional diversity. In this linear mixed-effects model, data from the two years were combined; functional diversity was the dependent variable, Shannon-Weiner diversity was the independent variable, and study year, study plot identity, and habitat category were included as random effects.

While a direct comparison of diversity metrics provides information on how habitat categories differ from one another, assessing whether observed differences are driven by stochastic or deterministic processes requires testing null models (Chase and Myers 2011). Here, we generated random bee communities for each study plot that have species richness and Shannon-Weiner diversity equivalent to their respective observed communities in order to test whether observed differences in functional diversity are simply due to underlying differences in species richness (see Chapter 1 of this

dissertation). In this analysis, we generated random communities for each study plot (used R package *vegan*) by first permuting observed species-level individual abundances across all species within the species pool for the study year in question (n = 500 permutations), and then rarefying each randomly permuted community to the lowest plot-level bee abundance recorded each year (n = 20 iterations). This permutation procedure resulted in 10,000 random communities for each study plot in each year. We then assembled 100,000 datasets by randomly selecting one permuted community from each study plot, compared the FDis scores of reserve and fragment plots via two-sample *t*-tests, and extracted the test statistic (*t*-value) of each comparison. Finally, we compared the test statistic of our empirical dataset against the null distribution of test statistics to assess the frequency with which null datasets yielded FDis differences between reserves and fragments that equal or exceed those observed in our empirical dataset.

Taxonomic and functional beta diversity and assemblage composition: To assess spatial beta diversity among plots, we used analyses of multivariate dispersion (Anderson et al. 2006, 2011), which compare habitat categories with respect to the degree of compositional similarity among their constituent study plots. Multivariate dispersion is calculated (using R package *vegan*) by first performing non-metric multidimensional scaling (NMDS) ordinations based on all combinations of pairwise among-plot dissimilarity in bee taxonomic or functional composition (weighted by relative abundances of species), and then comparing the non-metric distances of plots from the centroids of their respective habitat categories via a permutation test (Anderson et al. 2006). In our analysis of taxonomic beta diversity, pairwise among-plot dissimilarity was calculated as the abundance-weighted Bray-Curtis distance between each pair of plots. In

our analysis of functional beta diversity, we first calculated the coordinates of each plot's abundance-weighted functional centroid in multivariate trait space (Boersma et al. 2016), and then calculated pairwise among-plot dissimilarity as the non-metric distances between these functional centroids. We performed 10,000 permutations for calculations of both functional and taxonomic beta diversity.

In addition to examining beta diversity among plots within each habitat category, we also assessed whether reserves and fragments differed from each other with respect to the taxonomic and functional assemblage composition of their bee faunas. To accomplish this comparison, we performed permutational multivariate analyses of variance (PERMANOVA, Anderson 2001) on the same pairwise among-plot dissimilarity scores used to calculate beta diversity, described above, with 10,000 permutations (using R package *vegan*). Lastly, we also performed a Mantel test to examine the relationship between taxonomic and functional composition, based on the same pairwise distance matrices discussed above.

Unbalanced designs such as that used in year 2012 of our study are known to introduce bias into PERMANOVA tests when within-group heterogeneity is unequal among habitat categories (Anderson and Walsh 2013). Thus, to aid in the interpretation of our results, we also performed tests of beta diversity and assemblage composition for the 2012 dataset on random subsamples of 6 fragment plots (iterated 1,000 times) and examined the proportion of results in which the findings of the subsamples agreed with those of the full dataset with all 11 fragment plots included.

Drivers of variation in taxonomic and functional diversity and composition: To assess the drivers underlying differences between reserves and fragments with respect

to taxonomic and functional diversity and composition, we performed two additional analyses. First, we used two-sample *t*-tests to compare bee assemblages in reserve and fragment plots with respect to the relative representation of each functional trait. In this analysis, we used the plot-level mean average for intertegular length (i.e., the proxy for body size), and the plot-level proportional representation by each categorical or binary state for all other traits. Proportion data were logit-transformed prior to analysis as recommended by Warton and Hui (2011), and all calculations were weighted by the relative abundance of each species. Second, we performed an indicator species analysis (using R package *indicspecies* (De Cáceres and Legendre 2009) to identify bee species or functional groups associated with each habitat category. We used the Indval.g association index (De Cáceres et al. 2010) in the indicator analysis to account for the unbalanced sampling design in 2012. To assign bee species to functional groups, we constructed a dendrogram (using R package *FD*) of all bee species collected in the study (i.e., including species only collected via netting) using hierarchical clustering based on functional trait data (Petchey and Gaston 2006). We used Ward's algorithm to perform hierarchical clustering (Ward 1963), and assigned bees into 25 functional groups based on their positions in the dendrogram. Functional group membership of each species is given in Table 2-S1.

Geographical range sizes: For bee taxa identified to described species, we calculated their geographical range size based on our field data and the database of the Bee Research Laboratory of the United States Department of Agriculture. This database includes specimens collected from throughout the United States, Canada, and Mexico; and represents one of the most comprehensive and unbiased databases of bees in our

study region. These data enabled us to calculate range size for 171 bee species (Table 2-S1); range size calculations were not possible for species with too few specimen records (18 species) or taxa not identified to described species (39 morphospecies). Using geographical information systems (GIS) analyses available via ArcGIS (ESRI 2017) and QGIS (QGIS Development Team 2017), we mapped individual records of each bee species and constructed concave (alpha-shape) polygons (Edelsbrunner et al. 1983) bounding the set of location data points for each species. Range size for each species was calculated as the internal area of each species' concave polygon, which yields the smallest area that encompasses the set of location data points for each species and allows for more accurate determination of species range size compared to convex polygons. We then calculated the average range size of all bee individuals at each study plot ($\log_{10}$-transformed to improve normality), and compared average range sizes between reserve and fragment plots using two-sample *t*-tests.

Accounting for non-independence of traits resulting from phylogeny: Functional trait distribution among species is often influenced by phylogeny (Peterson 1999, Webb et al. 2002), including in bees (Bartomeus et al. 2013). Thus, to aid in the interpretation of our results in view of phylogenetic signals present in our data, we quantified the variation in each trait attributed to each major taxonomic rank by constructing nested generalized linear mixed-effects models (Pagel and Harvey 1988). In these models (constructed using R package *glmmADMB* (Fournier et al. 2012)), the value of each trait is the dependent variable, and taxonomic ranks (family, subfamily, and genus) were included as random effects (Table 2-2). Additionally, relationships between traits and evolutionary history also result in mutual correlation among traits; thus, we also

constructed a Spearman rank correlation matrix of traits (Table 2-3). In constructing nested linear models and the correlation matrix, lecty was converted from a categorical variable (Table 2-1) into a quantitative variable corresponding to diet niche breadth (cleptoparasitic = 1, oligolectic = 2, mesolectic = 3, polylectic = 4) to aid in model fitting.

RESULTS

In both years, fragments harbored bee assemblages with lower Shannon-Weiner diversity (Fig. 2-1A, on average 29% lower) and functional dispersion (Fig. 2-1B, on average 20% lower) compared to reserves. Functional and taxonomic diversity were closely related to each other (Fig. 2-1C, $t_{14.9} = 5.73$, $P < 0.001$), and indeed, null models revealed that the reduced functional diversity in fragments is explained by reduction in taxonomic diversity ($P = 0.13$ in 2011, $P = 0.081$ in 2012, n = 100,000 permutations).

Despite strong differences in spatial alpha diversity, reserves and fragments did not differ with respect to either taxonomic or functional beta diversity in either year (Fig. 2-2). However, in both years, reserves and fragments harbored distinct bee faunas with respect to both functional and taxonomic assemblage composition (Fig. 2-2). Functional and taxonomic composition were closely related to each other (Mantel's $r = 0.82$, $P = 0.001$ in 2011; Mantel's $r = 0.80$, $P = 0.001$ in 2012). Results from the reanalysis of the 2012 dataset rarefied to 6 fragment plots were in qualitative agreement with those of the full dataset (Fig. 2-S1) for both measures of beta diversity (100% of cases for both measures) and assemblage composition (70% of cases for taxonomic composition, 68% for functional composition). Given these results, differences in assemblage composition

detected between reserves and fragments in 2012 are unlikely to be artefacts of the unbalanced design.

When bee assemblages in reserves and fragments are compared with respect to the relative representation of individual traits, fragments harbored relatively fewer oligolectic bees, more polylectic bees, more bees that excavate or construct their own nests, and more bees active in the middle and late flight seasons (Fig. 2-3, Table 2-4). Indicator analyses identified 12 bee species and five functional groups as being associated with reserves, and none associated with fragments (Table 2-5). Indicator species associated with reserves tended to be those that excavate nests underground and have periods of activity spanning at least two flight seasons (i.e., early to middle or middle to late season). However, indicator species exhibited a range of states with respect to the remainder of the functional traits (Table 2-5). Similarly, the functional groups identified included bee species exhibiting a diversity of functional characteristics (Table 2-5).

In 2012, fragment plots harbored bee assemblages with larger average range sizes compared to reserve plots (Fig. 2-4). While this comparison was not quite statistically significant at the $\alpha = 0.05$ level in 2011, there was a trend in the same direction as that detected in 2012 (Fig. 2-4).

DISCUSSION

Across two years of sampling, we found that study plots in fragments harbored bee assemblages with reduced plot-level functional diversity and distinct functional composition compared to those in reserves. Changes in functional diversity and

composition were closely related to declines and shifts in taxonomic diversity and composition. While we found strong evidence for non-random patterns of species loss, such patterns of loss was insufficient to cause landscape-level taxonomic or functional homogenization in the fragments. Null model analyses and correlational analyses also demonstrate that the loss of bee functional diversity can be explained by loss of bee taxonomic diversity. Taken together, these findings suggest that ecological filtering contributes to the restructuring of bee assemblages, but is not the main driving force of bee diversity loss in habitat fragments in our system.

The strongest support for the importance of ecological filtering in our system is the detection of multiple indicator species and functional groups that appear particularly susceptible to fragmentation, typical of "winner-loser" dynamics found in modified landscapes (Lôbo et al. 2011, Rader et al. 2014). Also typical of "winner-loser" dynamics, we found a number of species that are present at all study plots, most of which are eusocial species in the tribe Halictini, which are known to be tolerant of habitat fragmentation (e.g., Hinners et al. 2012). However, unlike systems where small numbers of "winner" taxa or functional groups dominate modified landscapes (e.g., Tabarelli et al. 2012), indicator analyses revealed no such "winner" species or functional groups associated with fragments. Our finding only indicator taxa associated with reserves suggests that ecological filtering indeed leads to the exclusion of certain "loser" taxa and functional groups from fragments, but not to such an extent that the bee assemblages become numerically dominated by groups of disturbance-tolerant species that thrive in altered habitats. In fact, the loss of "loser" taxa seems to largely underlie the detected directional shifts in both taxonomic and functional measures of assemblage composition

(Fig. 2-2); simply removing the 12 indicator species from the analyses nullifies the significant differences detected between reserves and fragments with respect to both taxonomic and functional composition (Table 2-S3).

Evaluating differences between bee faunas in reserves and fragments one trait at a time revealed several differences between reserves and fragments, but only two that remained statistically significant after correction for multiple comparisons (Table 2-4). Preferential loss of specialists in modified environments has been documented in many taxa (Henle et al. 2004), including bees (Cane et al. 2006). The increased relative abundance of late-season active bees observed in the present study has also been reported in at least one other system (Wray and Elle 2015) in which bees in modified landscapes have enhanced access to anthropogenic sources of floral resources during periods of relative resource dearth (but see (Forrest et al. 2015). In our system, it is likewise plausible that late-season bees in fragments are able to thrive by foraging on floral resources in the irrigated urban matrix surrounding fragments.

The increase in average range size of bees inhabiting fragments also reveals the role of ecological filtering in structuring bee assemblages our system. Range size is not a functional trait *per se*, but it does serve as a proxy for an important ecological function that remains difficult to quantify: overall niche breadth (Brown 1984, Slatyer et al. 2013). While many studies on bees focus on lecty as the main metric for niche breadth (e.g., Williams et al. 2010), selectivity of nesting substrates (Westrich 1996), phenological flexibility (Rafferty et al. 2015), and physiological tolerance to abiotic conditions (Classen et al. 2015) may all influence how bee species respond to the addition of novel ecological filters. Our results suggest that bees in fragments tend to be those that are

capable of surviving in a greater number of ecological contexts compared to bees in reserves, consistent with the view ecological filters present in habitat fragments exclude species that are more narrowly adapted to the unique local ecosystems. Such replacement of endemics by geographically widespread species has been observed in other systems impacted by habitat alterations (McKinney and Lockwood 1999, Scott and Helfman 2001), and may be an important force driving reductions in ecological complexity across large spatial scales.

Given that bee assemblages in fragments exhibited strong reductions in both taxonomic and functional alpha diversity as well as distinct taxonomic and functional composition compared to reserves, it is noteworthy that reserves and fragments did not differ with respect to either taxonomic or functional beta diversity. Reduced beta diversity is associated with biotic homogenization, which is a hallmark of ecological filtering resulting from anthropogenic disturbance (Olden and Rooney 2006). Biotic homogenization resulting from land use change has been found across many taxa (Olden and Rooney 2006), including pollinators (Quintero et al. 2010, Gámez-Virués et al. 2015). However, unlike other systems in which anthropogenic impact is dominant and pervasive, such as in cases where intensive agriculture generated highly simplified landscapes (Karp et al. 2012, Liu et al. 2016), the habitat fragments we selected in our study were comparable to our natural reserve sites with respect to both the diversity and the composition of native, insect-pollinated plant assemblages, at least at the scale of our one-hectare study plots (see Chapter 1 of this dissertation, Appendix 1-1). The local plant community determines the composition of bee assemblages to a large extent (Michener 1979, Westrich 1996). Thus, given that our fragment plots retained relatively intact plant

assemblages, it is perhaps unsurprising that bee assemblages therein had not converged to a subset of taxa that thrive in altered habitats (Quintero et al. 2010). As with the findings of (Tscharntke et al. 2002), robust beta diversity among fragments may result from underlying heterogeneity in the habitat characteristics of our fragment plots.

Taxonomic and functional diversity are often positively related to each other (Flynn et al. 2009, Cadotte et al. 2011), but the two measures of diversity are related to each other in complex ways and may be independently impacted by habitat modifications (Mayfield et al. 2010). These complex relationships may explain our null model analysis, wherein the reduction in functional diversity in fragments did not differ from expectation under stochastic species loss despite our detecting multiple "loser" functional groups that suffer declines in fragments. While the parallel declines in taxonomic and functional diversity we detected in fragments via both null model and correlation analysis (Fig. 2-1C) may indeed indicate stochastic loss of species (Flynn et al. 2009, Mayfield et al. 2010), such a pattern could also arise from non-random loss of species whose functional traits have dispersions comparable to those lost due to random removal of species in the null model. Our finding that the "loser" species and functional groups associated with reserves varied with respect to every functional trait measured (Table 2-5) lends support to the latter mechanism. Alternatively, the apparent non-uniformity in the functional traits of "loser" taxa may result from our not measuring some other functional traits that may be shared among these taxa. For example, if dispersal (e.g., Bommarco et al. 2010) is the main driver of bee assemblage composition in fragments, a functional trait that strongly influences the likelihood of dispersal across the urban matrix may be largely responsible for interspecific variation in likelihood of local extirpation from fragments. Irrespective

of the mechanism underlying the parallel declines in taxonomic and functional diversity, our null model and correlational analysis results suggest that in our system, managing habitats in such a way as to preserve taxonomic diversity may be an effective way to preserve functional diversity (Cadotte et al. 2011).

We uncovered significant phylogenetic conservatism (Peterson 1999) in the functional traits we measured (Table 2-2), which likely contributed to the numerous correlations detected among traits (Table 2-3), a pattern also reported in other studies involving bee functional traits (Williams et al. 2010, Forrest et al. 2015). Given that phylogenetic conservatism in functional traits can shape the ecology and distribution of bee species in a landscape (Pellissier et al. 2013), our findings must be interpreted in the context of fragmentation impacting bees at the level of higher taxa. However, since analyses at the level of genera yielded qualitatively similar results (Table 2-S2), the overall patterns we report are unlikely to be driven by a few species-rich groups that respond especially strongly to fragmentation. The detection of indicator species belonging to three families and indicator functional group members belonging to five families (out of six families total; the family not represented by functional group members contained a single rare species) also suggests that impacts of fragmentation are not limited to certain clades of bees. Phylogenetic relationships among bee taxa are a subject of ongoing research, even at the level of higher taxa (Hedtke et al. 2013, Litman et al. 2016). Once accepted phylogenies become available for bee taxa occurring in our system, it would be instructive to quantify the extent to which evolutionary relationships among taxa contribute to our findings, and the implications fragmentation may have on the evolutionary trajectory of bee faunas as time progresses.

The maintenance of both taxonomic and functional beta diversity in our studied fragments argues for the preservation of each individual fragments of CSS habitat, despite the fact that fragments as a whole share the absence of sensitive “loser” bee taxa and functional groups. Our results suggest that each fragment preserves its own distinctive subset of the bee faunas formerly present in the regional species pool, and thus by extension, their ecological interactions with other taxa such as plants, parasitic or commensal invertebrates (e.g., Michener 2007, McFrederick et al. 2013), and microbes (e.g., Ushio et al. 2015). High levels of heterogeneity in assemblage composition among fragmented habitat remnants have also been documented in other systems (Tscharntke et al. 2002, Sfair et al. 2016); in such systems, the cumulative species pool of compositionally divergent fragments may equal or exceed the species pools of unfragmented habitat. Beta diversity as a result of habitat heterogeneity is a strong driver of local and regional diversity of pollinators (Norfolk et al. 2015, Rollin et al. 2015) and organisms in general (Tscharntke et al. 2012). In our system, bee faunas occupying habitat fragments embedded in a heterogeneous landscape do not exceed or equal those in larger natural reserves with respect to taxonomic or functional diversity, but nevertheless represent valuable units of conservation that may each exhibit unique community-level evolutionary trajectories with time if properly preserved. In fact, of the 216 species collected in the study (including specimens collected via both aerial net and bowl traps), 40 were unique to fragments (including 25 singletons), while 74 were unique to reserves (including 27 singletons). That said, the decrease in plot-level functional diversity in habitat fragments still represents a conservation challenge with respect to both the

functionality and the resilience of bee faunas (Blüthgen and Klein 2011, Forrest et al. 2015), highlighting the importance of preserving large, intact areas of scrub habitat.

CONCLUSIONS

We demonstrated that ecological filtering in fragmented scrub habitats caused shifts in the taxonomic and functional composition of bee faunas as a result of a loss of sensitive bee taxa and an increase in the relative abundance of geographically widespread bee species. However, filtering was not sufficiently strong to reduce functional diversity beyond that expected under random species loss, and bee faunas in fragments retained taxonomic and functional beta diversity among plots. Future studies that can quantitatively partition the relative contribution of deterministic and stochastic processes in driving taxonomic and functional diversity loss will shed light on the factors influencing community reassembly in structurally intact but isolated fragments of well-preserved natural habitat.

ACKNOWLEDGMENTS

We gratefully acknowledge the taxonomic assistance of J. Gibbs, T. Griswold, M. Orr, Z. Portman, M. Rightmyer, S. Sipes, R. Thorp, K. Wright, and D. Yanega, as well as field and laboratory assistance of S. Fung, W. Wen, R. Graham, C. Johnson, J. May, and S. Plesim. We also gratefully acknowledge K. Boersma, B. Novak-Pilch, A. Ranganath, and S. Rifkin for assistance with data analysis, as well as N. Jones, J. Kohn, L. Levin, J. Minster, J. Nieh, K. Roy, and J. Shurin for providing constructive feedback on our manuscript. Additionally, we thank H. Ikerd, T. Griswold, and D. Yanega for providing

databases from the UC Riverside Entomological Museum and the USDA Bee Research Laboratory. This work was performed in scrub habitats managed by the University of California Natural Reserve System (Scripps Coastal Reserve and Elliott Chaparral Reserve), Open Space Parks in San Diego County (cities of Chula Vista, La Mesa, and San Diego), and the Otay-Sweetwater Unit of the San Diego National Wildlife Refuge. This work was funded by the UC San Diego Academic Senate Bridge Fund Grant BIO088B, the NSF Doctoral Dissertation Improvement Grant DEB-1501566, the National Geographic Society Young Explorer Grant 9014-11, the Mildred E. Mathias Graduate Student Research Grant from the University of California Reserve System, the Jeanne Marie Messier Memorial Endowed Fund, and Sigma Xi Grant in Aid of Research G20111015158245.

Chapter 2, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Ascher, John S.; Davids, Jessica A.; Holway, David A. The dissertation author was the primary investigator and author of this manuscript.

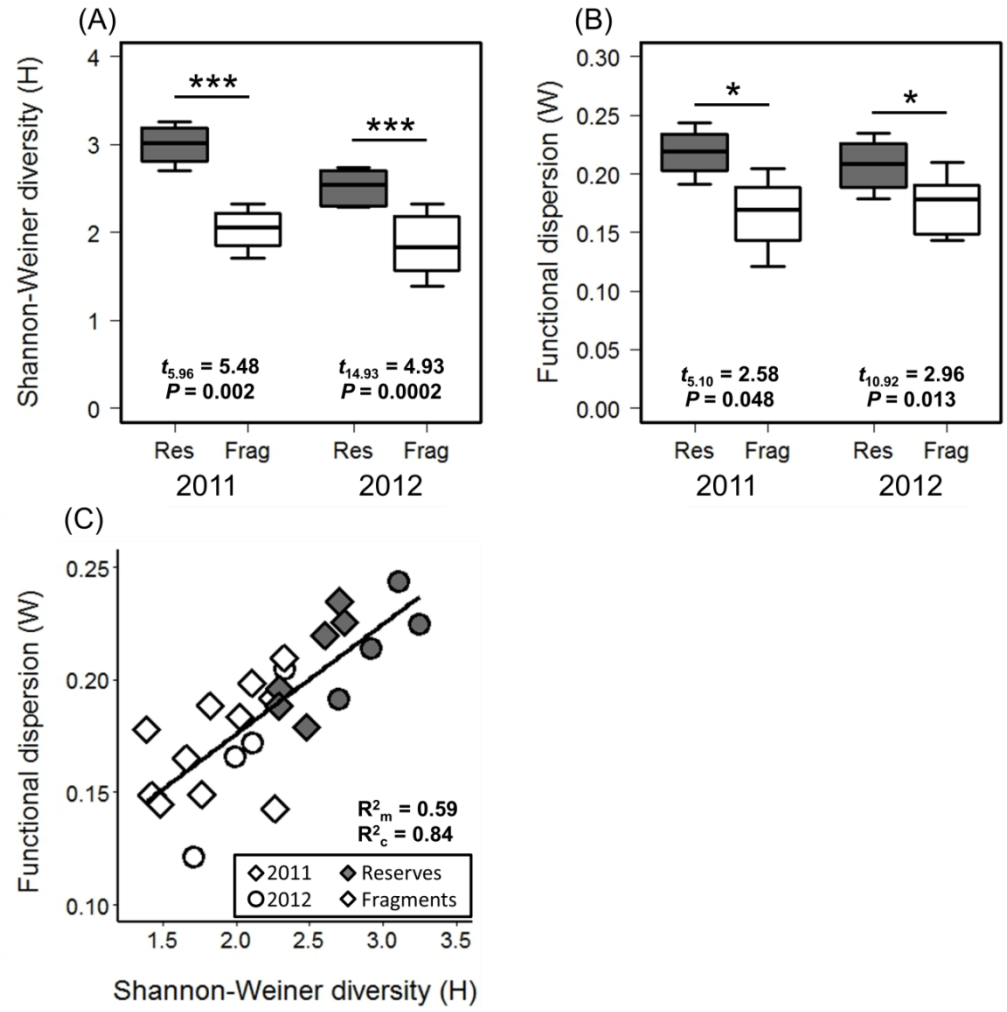

Figure 2-1. Alpha diversity of native bees in reserve and fragment plots. Plots (A) and (B) show rarefied Shannon-Weiner diversity and rarefied functional dispersion (FDis, a metric of functional diversity), respectively, with data from the two study years analyzed separately. Boxes show central 50% of data and median; whiskers show quantiles ± 1.5 × interquartile range, or most extreme values of data, whichever is closest to median. Plot (C) shows relationship between taxonomic and functional diversity, with data from both study years and both habitat categories analyzed together. Marginal and conditional R^2 values were calculated for linear mixed-effects models with study year, habitat category, and study plot identity as random effects.

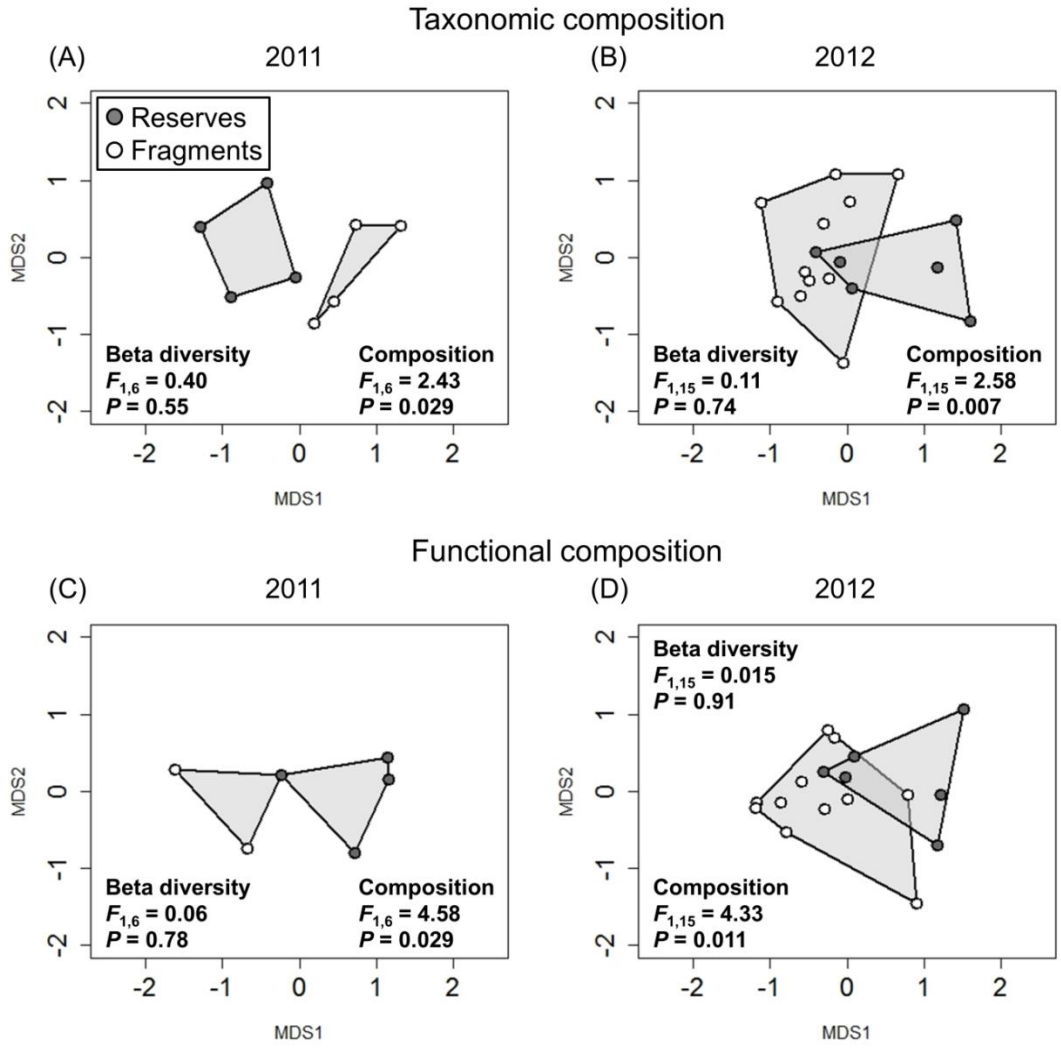

Figure 2-2. Non-metric multidimensional scaling (NMDS) ordination plots of bee assemblages in reserve plots (gray circles) and fragment plots (white circles), with respect to taxonomic (A-B) and functional (C-D) composition. Beta diversity is visualized as the degree of dispersion among plots within each habitat category, while assemblage composition is visualized as the displacement of each habitat category's set of plots relative to each other. Ordinations were constructed based on dissimilarity between each pair of plots with respect to Bray-Curtis distances for taxonomic composition and distances between functional centroids of plots (Boersma et al. 2016) for functional composition.

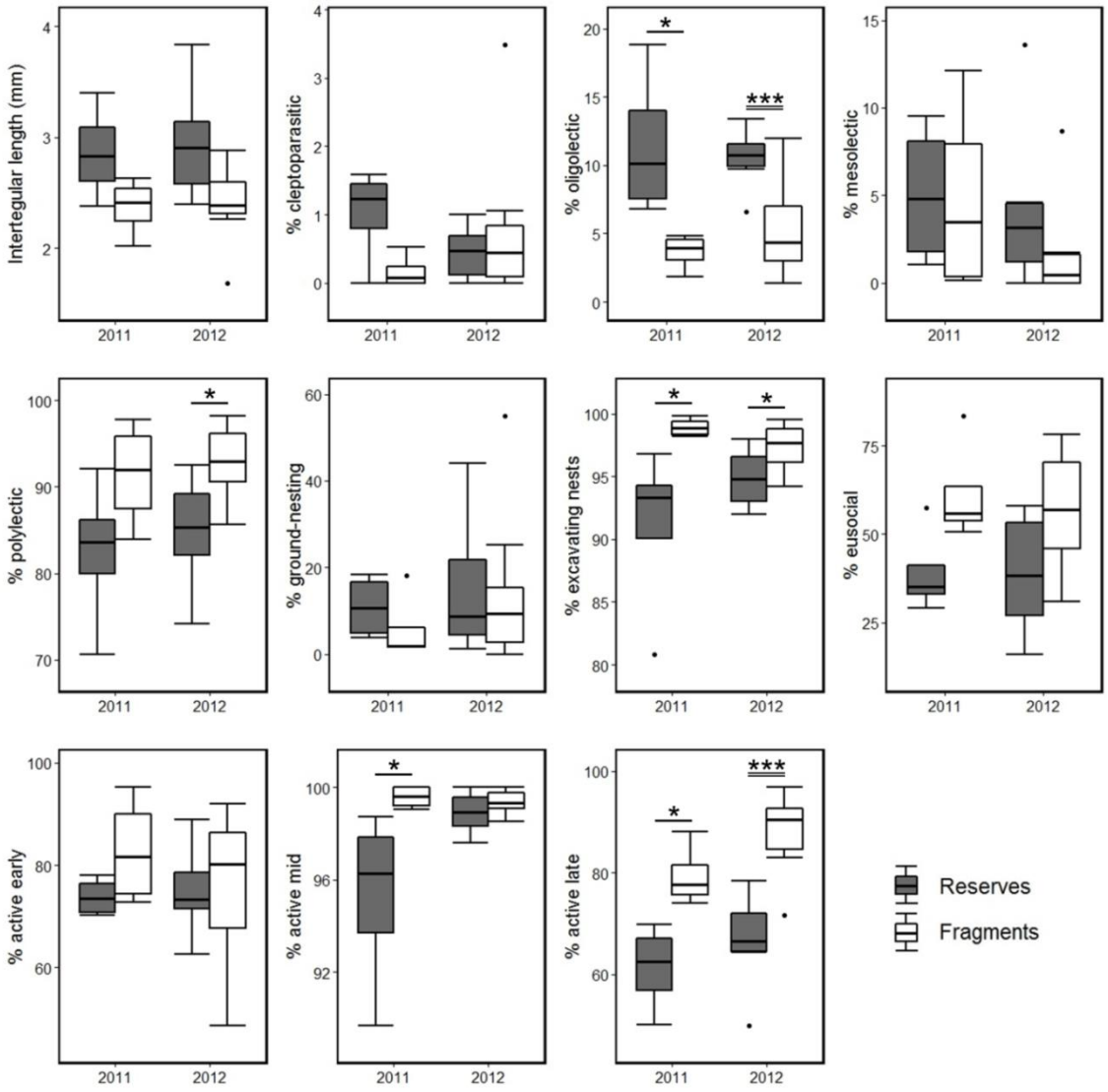

Figure 2-3. Bee faunas in reserve and fragment plots with respect to the relative representation of each functional trait. Boxes and whiskers are as in Fig. 2-1. * $P < 0.05$; ** $P < 0.01$; ***$P < 0.005$; asterisks accompanied by double underlines were statistically significant at the $\alpha = 0.05$ level after Benjamini-Hochberg false discovery rate correction for multiple comparisons.

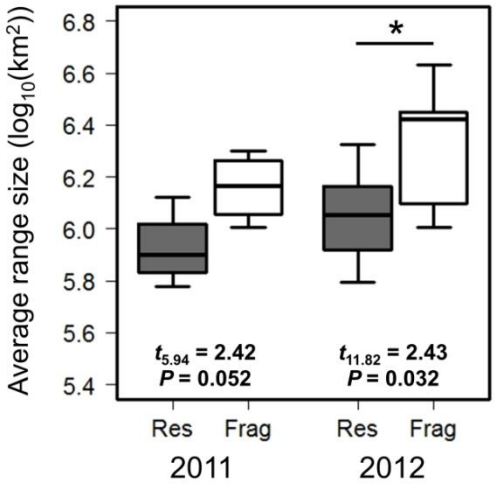

Figure 2-4. Average range sizes of bee assemblages in reserves and fragments. Boxes, whiskers, and asterisks are as in Fig. 2-1.

Table 2-1. Functional traits of native bees used in analyses of functional diversity. Variable type refers to the classification of each trait in the calculation of functional dispersion (FDis).

Trait	Variable type	Description
Body size	Continuous	Mean intertegular length (mm) of 3-4 randomly selected females per species
Lecty	Categorical (4 categories)	Cleptoparasitic = limited to pollen resources of their host bees Oligolectic = specialized on a single plant family Mesolectic = restricted to or strongly preferring pollen from < 5 plant families Polylectic = accepting pollen from ≥ 5 plant families
Nest location	Semi-quantitative (After Forrest et al. 2015), weighted at 0.5	0 = nesting belowground 0.5 = opportunistically nesting either above or belowground 1 = nesting above ground
Nest building	Binary, weighted at 0.5	0 = occupying various pre-existing cavities 1 = actively excavating or constructing nest in soil, plant stems, or other substrates
Sociality	Binary	0 = solitary, no reproductive division of labor (i.e., each female produces own offspring) 1 = eusocial, with reproductive division of labor
Flight season	Three binary traits (After Tonietto et al. 2016)	Presence / absence in each of three season categories based on data from regional database
Early	Binary, weighted at 0.5	0 = not active; 1 = active Mar-Apr
Middle	Binary, weighted at 0.5	0 = not active; 1 = active May-Jun
Late	Binary, weighted at 0.5	0 = not active; 1 = active Jul-Aug

Table 2-2. Variance of functional traits for the taxonomic ranks of family, subfamily, and genus. Variance is calculated using nested generalized linear mixed-effects models.

Trait	Model	Family	Subfamily	Genus	Residual
Body size	Gaussian	0.05	1.03	1.43	0.6
Lecty	Gaussian	0	0.45	0.83	0.61
Nest location	Gaussian	0	1.28	0.18	0.42
Nest building	Binomial	0.2	272.1	41.54	N/A
Sociality	Zero-inflated negative binomial	0.02	2.95	49.69	N/A
Season (Early)	Binomial	0	0.002	1.44	N/A
Season (Middle)	Binomial	0	0	2.6	N/A
Season (Late)	Binomial	0	0	2.25	N/A

Table 2-3. Spearman rank correlations among functional traits. Coefficients in bold are statistically significant at the α = 0.05 level after Benjamini-Hochberg false discovery rate correction for multiple comparisons. * P < 0.05; ** P < 0.01; ***P < 0.005.

Traits	Lecty	Nest location	Nest building	Sociality	Season (Early)	Season (Middle)	Season (Late)
Body size	0.12	-0.16*	-0.04	**-0.23****	-0.11	-0.18*	0.08
Lecty		-0.15*	**0.34*****	**0.39*****	0.07	**0.22***	0.18*
Nest location			**0.59*****	**0.24*****	-0.06	-0.14*	-0.02
Nest building				**0.23****	0	-0.01	0.11
Sociality					0.1	0.14*	0.08
Season (Early)						-0.16*	**-0.52*****
Season (Middle)							0.16*

Table 2-4. Results of two-sample *t*-tests comparing bee assemblages in reserve and fragment plots with respect to the relative representation of each functional trait. *P* values in bold are statistically significant at the α = 0.05 level after Benjamini-Hochberg false discovery rate correction for multiple comparisons.

	2011			2012		
Trait	DF	*t*	*P*	DF	*t*	*P*
Mean intertegular length	4.97	1.93	0.11	7.13	2.24	0.059
% cleptoparasitic	5.4	1.51	0.19	10.77	-0.34	0.74
% oligolectic	5.83	3.51	0.013	14.49	4.12	**0.001**
% mesolectic	4.53	0.68	0.53	10.43	1.61	0.14
% polylectic	5.31	-1.69	0.15	13.14	-3.09	0.009
% nesting underground	5.33	1.4	0.22	14	0.63	0.54
% excavating nests	5.92	-3.61	0.01	14.76	-2.47	0.026
% eusocial	5.44	-2.19	0.08	9.4	-2.07	0.067
% active early season	3.27	-1.53	0.22	14.34	-0.27	0.79
% active mid season	4.68	-3.01	0.032	9.95	-0.75	0.47
% active late season	5.86	-3.26	0.018	14.3	-5.28	**0.0001**

Table 2-5. Bee species and functional groups exhibiting significant associations with scrub reserve plots in each study year. In cases where traits are not uniform across species within a functional group, the modal average is reported. See Table 2-S1 for functional group membership. For flight seasons, 0 indicates not active and 1 indicates active for the season in question. * $P < 0.05$; ** $P < 0.01$.

Group	Indicator value	ITL (mm)	Lecty	Nest location	Nest building	Sociality	Flight seasons Early	Mid	Late
2011 species									
Augochlorella pomoniella	0.95*	2.57	Polylectic	Underground	Excavate	Eusocial	0	1	1
Diadasia laticauda	0.98*	4.15	Oligolectic	Underground	Excavate	Solitary	0	1	1
Lasioglossum robustum	1.00*	3.29	Polylectic	Underground	Excavate	Eusocial	1	1	0
Melissodes communis	0.98*	4.68	Polylectic	Underground	Excavate	Solitary	0	1	1
Melissodes plumosa	1.00*	3.86	Oligolectic	Underground	Excavate	Solitary	0	1	1
2012 species									
Anthidium jocosum	0.85**	3.58	Polylectic	Mixed	Rent	Solitary	1	1	0
Anthophorula torticornis	0.86*	2.67	Polylectic	Underground	Excavate	Solitary	1	1	0
Ashmeadiella foveata	0.66*	2.86	Polylectic	Underground	Excavate	Solitary	1	1	1
Augochlorella pomoniella	0.92**	2.57	Polylectic	Underground	Excavate	Eusocial	0	1	1
Dianthidium dubium	0.76*	3.29	Polylectic	Aboveground	Excavate	Solitary	0	1	1
Dianthidium pudicum	0.85**	3.39	Polylectic	Aboveground	Excavate	Solitary	0	1	1
Eucera dorsata	0.76*	5.01	Polylectic	Underground	Excavate	Solitary	1	1	0
Eucera tricinctella	0.85**	5.11	Polylectic	Underground	Excavate	Solitary	1	1	0
Lasioglossum robustum	0.76*	3.29	Polylectic	Underground	Excavate	Eusocial	1	1	0
Megachile cf. *seducta*	0.76*	5.29	Oligolectic	Mixed	Rent	Solitary	1	1	0
Melissodes communis	0.92**	4.68	Polylectic	Underground	Excavate	Solitary	0	1	1
2011 functional groups									
Group 2 (14 spp.)	0.96*	1.14 - 4.29	Oligolectic	Underground	Excavate	Solitary	1	0	0
2012 functional groups									
Group 4 (6 spp.)	0.95**	2.67 - 6.72	Polylectic	Underground	Excavate	Solitary	1	1	0
Group 20 (12 spp.)	0.85*	1.53 - 5.00	Polylectic	Aboveground	Rent	Solitary	1	1	0
Group 22 (5 spp.)	0.95**	1.72 - 2.86	Polylectic	Underground	Excavate	Eusocial	0	1	1
Group 24 (12 spp.)	0.80*	1.72 - 5.29	Oligolectic	Mixed	Rent	Solitary	1	1	0

APPENDIX 2-1. Supplemental data and analyses.

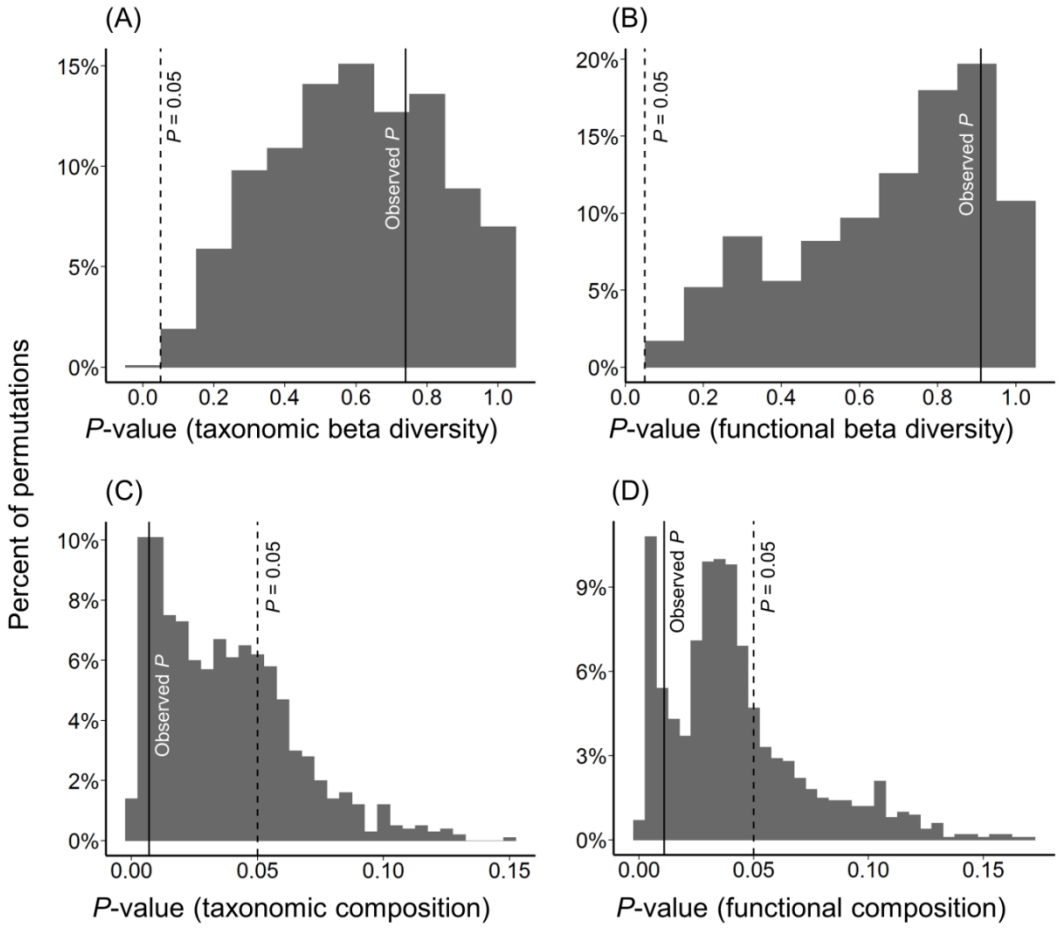

Figure 2-S1. Distribution of *P*-values from comparisons of beta diversity (A-B) and assemblage composition (C-D) between reserve and fragment plots in the 2012 dataset (n = 1,000 permutations). Each comparison consists of all reserve plots (n = 6) and a random subsample of n = 6 fragment plots (out of a total of n = 11 fragment plots) to achieve a balanced design. For both taxonomic and functional measures of beta diversity and assemblage composition, results of rarefied analyses are in qualitative agreement with results of the full dataset, and therefore support the conclusions drawn from the main analyses.

Table 2-S1. Functional traits, functional group membership, and range size of each bee species and morphospecies included in our study. Columns are as follows. ITL = intertegular length in mm. Lecty: cleptoparasitic (C), oligolectic (O), mesolectic (M), polylectic (P). NL = nest location: ground (G), cavity (C), mixed (M). NB = nest building: excavating (E), renting (R). Soc. = sociality: eusocial (E), solitary (S). Flight season: early (E; March and April), mid (M; May and June), late (L; July and August). FG = functional group membership calculated via hierarchical clustering based on functional traits. RS = range size in km^2.

						Flight season				
Species	ITL	Lecty	NL	NB	Soc.	E	M	L	FG	RS
Andrenidae										
Ancylandrena atoposoma	4.00	O	G	E	S	1	1	0	1	124607
Andrena anatolis	2.29	O	G	E	S	1	0	0	2	N/A
Andrena atypica	2.15	P	G	E	S	1	0	0	3	37184
Andrena auricoma	3.15	P	G	E	S	1	1	0	4	866725
Andrena candida	2.00	P	G	E	S	1	1	0	4	1331240
Andrena cerasifolii	3.72	P	G	E	S	1	1	0	4	1357480
Andrena gnaphalii	3.29	O	G	E	S	1	0	0	2	N/A
Andrena nr. *knuthiana*	2.72	P	G	E	S	1	0	0	3	N/A
Andrena osmioides	3.72	O	G	E	S	1	0	0	2	14289
Andrena pallidifovea	3.43	O	G	E	S	1	1	0	1	905067
Andrena parachalybea	3.29	O	G	E	S	1	0	0	2	N/A
Andrena piperi	2.43	P	G	E	S	1	0	0	3	1233060
Andrena prunorum	4.00	P	G	E	S	0	1	0	5	4543890
Andrena sola	4.15	P	G	E	S	1	0	0	3	1203730
Calliopsis mellipes	3.00	O	G	E	S	1	1	0	1	19845
Calliopsis obscurella	3.58	O	G	E	S	1	1	0	1	244163
Calliopsis pugionis	3.15	O	G	E	S	1	1	0	1	18065
Calliopsis rhodophila	2.67	M	G	E	S	1	1	1	6	657360
Macrotera tristella	1.39	M	G	E	S	1	1	0	6	138841
Panurginus sp. 1	2.00	M	G	E	S	1	0	0	6	N/A
Perdita californica	2.57	O	G	E	S	1	1	0	1	37118
Perdita claypolei	1.33	O	G	E	S	0	1	1	7	298633
Perdita eriastri	1.00	O	G	E	S	1	1	0	1	N/A
Perdita fieldi	1.72	O	G	E	S	0	1	0	8	29333
Perdita hirticeps	2.00	O	G	E	S	0	0	1	9	462667
Perdita interrupta	2.00	O	G	E	S	1	1	0	1	15123
Perdita minima	0.57	O	G	E	S	0	0	1	9	639674
Perdita rhois	1.47	P	G	E	S	0	1	1	10	37995
Perdita sp. 1	2.57	O	G	E	S	1	1	0	1	N/A

Species	ITL	Lecty	NL	NB	Soc.	Flight season E	M	L	FG	RS
Andrenidae (continued)										
Perdita trisignata	1.86	O	G	E	S	1	1	0	1	65699
Apidae										
Anthophora crotchii	6.86	P	G	E	S	1	0	0	3	352901
Anthophora curta	4.29	M	G	E	S	1	1	1	6	1946340
Anthophora urbana	5.29	P	G	E	S	0	1	1	10	1758790
Anthophorula nitens	2.82	P	G	E	S	0	1	1	10	357095
Anthophorula torticornis	2.67	P	G	E	S	1	1	0	4	86288
Bombus californicus	6.08	P	G	R	E	1	1	1	11	3476540
Bombus crotchii	7.11	P	G	R	E	1	1	1	11	166037
Bombus melanopygus	6.41	P	M	R	E	1	1	0	11	11429500
Bombus vosnesenskii	5.86	P	G	R	E	1	1	1	11	3028243
Brachynomada annectens	2.15	C	G	R	S	0	1	0	12	N/A
Ceratina acantha	2.04	P	C	E	S	1	1	1	17	723900
Ceratina arizonensis	1.04	P	C	E	S	1	1	1	17	556987
Ceratina nanula	1.86	P	C	E	S	1	1	1	17	2157520
Ceratina punctigena	3.00	P	C	E	S	1	1	1	17	62115
Diadasia australis	5.39	O	G	E	S	1	1	0	1	2286810
Diadasia bituberculata	5.72	O	G	E	S	1	1	0	1	104903
Diadasia diminuta	3.72	O	G	E	S	0	1	1	7	2140290
Diadasia laticauda	4.15	O	G	E	S	0	1	1	7	65980
Diadasia martialis	4.15	O	G	E	S	0	1	0	8	168516
Diadasia nitidifrons	4.33	O	G	E	S	1	1	0	1	664121
Diadasia ochracea	4.15	O	G	E	S	0	1	1	7	1621270
Diadasia opuntiae	5.29	O	G	E	S	1	1	0	1	22920
Diadasia rinconis	5.15	O	G	E	S	1	1	1	1	1631840
Epeolus compactus	2.86	C	G	R	S	0	1	1	15	976681
Eucera dorsata	5.01	P	G	E	S	1	1	0	4	101786
Eucera edwardsii	5.86	P	G	E	S	1	0	0	3	1549090
Eucera tricinctella	5.11	P	G	E	S	1	1	0	4	107339
Eucera virgata	5.29	P	G	E	S	1	0	0	3	123784
Habropoda depressa	6.72	P	G	E	S	1	0	0	3	196099
Habropoda tristissima	6.72	P	G	E	S	1	1	0	4	586725
Holcopasites ruthae	2.29	C	G	R	S	1	0	0	13	N/A
Leiopodus singularis	3.58	C	G	R	S	0	1	0	12	772326
Melecta edwardsii	5.86	C	G	R	S	1	0	0	13	71606
Melissodes communis	4.68	P	G	E	S	0	1	1	10	3724490
Melissodes lupina	3.86	O	G	E	S	0	1	1	7	5131850
Melissodes montana	5.01	O	G	E	S	0	1	0	8	1398150

Species	ITL	Lecty	NL	NB	Soc.	Flight season E	M	L	FG	RS
Apidae (continued)										
Melissodes sp. nov. 1	3.86	O	G	E	S	0	1	1	7	N/A
Melissodes paroselae	3.72	P	G	E	S	0	1	1	10	1890220
Melissodes personatella	4.29	O	G	E	S	0	1	1	7	123697
Melissodes plumosa	3.86	O	G	E	S	0	1	1	7	1141350
Melissodes stearnsi	3.58	O	G	E	S	0	1	0	8	937539
Melissodes tessellata	4.43	P	G	E	S	1	1	1	14	195801
Melissodes velutina	3.58	M	G	E	S	1	1	0	6	58002
Neopasites sp. 1	2.43	C	G	R	S	1	1	0	16	N/A
Neopasites sp. 2	1.14	C	G	R	S	1	0	0	13	N/A
Nomada sp. 1	2.29	C	G	R	S	1	0	0	13	N/A
Nomada sp. 2	1.43	C	G	R	S	1	0	0	13	N/A
Nomada sp. 3	3.58	C	G	R	S	1	1	0	16	N/A
Nomada sp. 4	2.29	C	G	R	S	0	1	1	15	N/A
Peponapis pruinosa	5.43	O	G	E	S	0	1	1	7	6081340
Tetraloniella davidsoni	5.58	O	G	E	S	1	1	0	1	52899
Tetraloniella sp. nov. 1	4.29	O	G	E	S	1	0	0	2	N/A
Tetraloniella pomonae	4.72	O	G	E	S	0	1	1	7	95928
Triepeolus californicus	3.58	C	G	R	S	0	1	1	15	N/A
Triepeolus matildae	3.15	C	G	R	S	0	1	0	12	N/A
Triepeolus melanarius	3.15	C	G	R	S	0	1	1	15	N/A
Triepeolus cf. *simplex*	2.72	C	G	R	S	0	1	1	15	N/A
Triepeolus utahensis	2.86	C	G	R	S	0	1	1	15	N/A
Triopasites penniger	1.86	C	G	R	S	0	1	0	12	406026
Xeromelecta californica	4.43	C	G	R	S	1	1	1	15	2305250
Xylocopa varipuncta	11.15	P	C	E	S	1	1	1	18	1535070
Colletidae										
Colletes aff. *deserticola*	4.00	O	G	E	S	0	1	1	7	N/A
Colletes intermixtus	3.72	P	G	E	S	0	1	0	5	1411300
Colletes louisae	4.00	P	G	E	S	1	1	0	4	986708
Colletes slevini	4.00	P	G	E	S	0	1	1	10	1382840
Colletes wootoni	3.72	P	G	E	S	0	1	1	10	789176
Hylaeus cookii	1.76	P	C	R	S	0	0	1	19	395499
Hylaeus episcopalis	2.29	P	C	R	S	1	1	0	20	2088270
Hylaeus mesillae	1.53	P	C	R	S	1	1	1	20	3219280
Hylaeus polifolii	1.76	P	C	R	S	1	1	1	20	460014
Hylaeus rudbeckiae	2.00	P	C	R	S	1	1	0	20	1010240
Hylaeus verticalis	2.29	P	C	R	S	1	1	0	20	2624490

Species	ITL	Lecty	NL	NB	Soc.	Flight season E	M	L	FG	RS
Halictidae										
Agapostemon texanus	3.43	P	G	E	S	0	1	1	10	11337700
Augochlorella pomoniella	2.57	P	G	E	E	0	1	1	22	4096840
Conanthalictus bakeri	1.72	O	G	E	S	1	0	0	2	325798
Dufourea aff. *sandhouseae*	2.00	O	G	E	S	1	0	0	2	N/A
Dufourea australis	2.57	O	G	E	S	1	1	0	1	62726
Dufourea brevicornis	2.00	O	G	E	S	1	0	0	2	N/A
Dufourea cf. *saundersi*	2.00	O	G	E	S	1	0	0	2	N/A
Dufourea mulleri	2.72	O	G	E	S	1	0	0	2	362566
Dufourea rhamni	1.72	O	G	E	S	1	0	0	2	29529
Dufourea scintilla	1.57	O	G	E	S	1	0	0	2	N/A
Halictus farinosus	3.90	P	G	E	E	1	1	0	21	3122610
Halictus ligatus	2.86	P	G	E	E	0	1	1	22	10789000
Halictus rubicundus	3.29	P	G	E	E	0	1	0	22	3189740
Halictus tripartitus	2.29	P	G	E	E	1	1	1	21	3769950
Lasioglossum (Dialictus) sp. 1	2.00	P	G	E	E	0	0	1	22	N/A
Lasioglossum (Dialictus) sp. 2	2.22	P	G	E	E	1	1	0	21	N/A
Lasioglossum (Dialictus) sp. 3	1.14	P	G	E	E	1	1	0	21	N/A
Lasioglossum (Evylaeus) sp. 1	1.86	P	G	E	E	1	1	0	21	N/A
Lasioglossum (Evylaeus) sp. 2	1.72	P	G	E	E	0	1	0	22	N/A
Lasioglossum (Evylaeus) sp. 3	2.43	P	G	E	E	1	0	0	21	N/A
Lasioglossum albohirtum	2.12	P	G	E	E	1	1	0	21	979715
Lasioglossum argemonis	2.40	P	G	E	E	1	1	0	21	60734
Lasioglossum brunneiventre	1.40	P	G	E	E	1	1	1	21	49761
Lasioglossum imbrex	1.57	P	G	E	E	1	1	1	21	3571
Lasioglossum incompletum	1.64	P	G	E	E	1	1	1	21	1197610
Lasioglossum knereri	1.90	P	G	E	E	1	1	0	21	N/A
Lasioglossum macroprosopum	1.86	P	G	E	E	1	1	0	21	N/A
Lasioglossum mellipes	3.43	P	G	E	S	1	1	0	4	289542
Lasioglossum microlepoides	1.93	P	G	E	E	1	1	1	21	1410470
Lasioglossum nevadense	1.47	P	G	E	E	1	1	0	21	490612
Lasioglossum nigrescens	1.69	P	G	E	E	1	1	0	21	25624
Lasioglossum nr. *nevadense*	1.83	P	G	E	E	1	1	1	21	N/A
Lasioglossum ovaliceps	2.29	P	G	E	S	1	1	0	4	1089940
Lasioglossum pacificum	3.29	P	G	E	S	1	1	1	14	1002080
Lasioglossum perparvum	1.19	P	G	E	E	1	1	0	21	134441
Lasioglossum petrellum	1.90	P	G	E	E	0	1	1	22	682855
Lasioglossum punctatoventre	2.04	P	G	E	E	1	1	0	21	978538
Lasioglossum robustum	3.29	P	G	E	E	1	1	0	21	1267

Species	ITL	Lecty	NL	NB	Soc.	Flight season E	M	L	FG	RS
Halictidae (continued)										
Lasioglossum sisymbrii	3.29	P	G	E	S	1	1	0	4	2768190
Lasioglossum titusi	3.29	P	G	E	S	0	1	1	10	917356
Micralictoides altadenae	1.14	O	G	E	S	1	0	0	2	N/A
Micralictoides chaenactidis	1.72	O	G	E	S	1	1	0	1	N/A
Micralictoides ruficaudus	1.57	O	G	E	S	1	0	0	2	233144
Sphecodes arvensiformis	3.43	C	G	R	S	1	1	0	16	510587
Sphecodes sp. 1	1.64	C	G	R	S	1	1	0	16	N/A
Sphecodes sp. 2	1.47	C	G	R	S	1	1	0	16	N/A
Sphecodes sp. 3	1.86	C	G	R	S	1	0	0	13	N/A
Sphecodes sp. 4	1.40	C	G	R	S	0	1	0	12	N/A
Sphecodes sp. 5	2.46	C	G	R	S	1	0	0	13	N/A
Sphecodes sp. 6	2.86	C	G	R	S	0	1	0	12	N/A
Sphecodes sp. 7	1.86	C	G	R	S	0	1	0	12	N/A
Sphecodes sp. 8	1.14	C	G	R	S	0	0	1	15	N/A
Sphecodes sp. 9	1.36	C	G	R	S	0	0	1	15	N/A
Sphecodes sp. 10	1.72	C	G	R	S	1	0	0	13	N/A
Sphecodes sp. 11	1.57	C	G	R	S	1	0	0	13	N/A
Megachilidae										
Anthidiellum notatum	4.29	P	M	E	S	0	1	1	17	4885070
Anthidium collectum	4.43	M	M	R	S	1	1	0	23	2676900
Anthidium illustre	5.86	P	M	R	S	1	1	0	20	639216
Anthidium jocosum	3.58	P	M	R	S	1	1	0	20	349575
Anthidium mormonum	4.43	P	M	R	S	0	1	0	19	2263580
Anthidium utahense	3.86	P	M	R	S	0	1	1	19	2160990
Ashmeadiella bucconis	3.15	O	C	R	S	0	1	1	24	4864690
Ashmeadiella californica	2.57	P	M	R	S	1	1	1	20	1708580
Ashmeadiella cubiceps	2.72	M	M	R	S	1	1	0	23	955276
Ashmeadiella foveata	2.86	P	G	E	S	1	1	1	14	898460
Ashmeadiella meliloti	2.29	P	M	R	S	0	1	1	19	2047930
Ashmeadiella rufitarsis	2.29	O	M	R	S	0	1	0	24	165288
Ashmeadiella salviae	2.43	M	M	R	S	1	1	0	23	192719
Ashmeadiella titusi	2.43	O	M	R	S	1	1	0	24	186513
Atoposmia copelandica	2.86	O	C	R	S	1	1	0	24	2114180
Atoposmia hemizoniae	3.86	O	C	R	S	0	1	0	24	N/A
Chelostoma californicum	2.57	O	C	R	S	1	1	0	24	258094
Chelostoma phaceliae	1.72	O	C	R	S	1	1	0	24	366770
Coelioxys sp. 1	4.58	C	C	R	S	0	0	1	25	N/A
Coelioxys sp. 2	4.00	C	C	R	S	0	0	1	25	N/A

Species	ITL	Lecty	NL	NB	Soc.	Flight season E	M	L	FG	RS
Megachilidae (continued)										
Dianthidium dubium	3.29	P	M	E	S	0	1	1	17	1579110
Dianthidium pudicum	3.39	P	M	E	S	0	1	1	17	3092180
Dioxys producta	3.29	C	M	R	S	1	1	0	16	1261880
Heriades occidentalis	2.43	P	C	R	S	0	1	1	19	210311
Hoplitis albifrons	4.29	P	C	R	S	0	1	0	19	11045900
Hoplitis cryptanthae	2.72	O	M	R	S	1	1	0	24	22291
Hoplitis fulgida	3.00	P	C	R	S	1	1	0	20	5709850
Hoplitis grinnelli	2.72	P	C	R	S	1	1	0	20	1576490
Hoplitis howardi	2.15	O	C	R	S	1	1	0	24	124433
Hoplitis hypocrita	4.29	P	C	R	S	1	0	0	20	3246130
Hoplitis remotula	2.72	O	M	R	S	1	1	0	24	218979
Hoplitis seminigra	2.00	O	M	R	S	1	1	0	24	138653
Hoplitis semirubra	2.72	O	M	R	S	1	1	0	24	47591
Megachile cf. *seducta*	5.29	O	M	R	S	1	1	0	24	N/A
Megachile coquilletti	5.29	P	M	R	S	0	1	1	19	2964710
Megachile fidelis	4.72	M	C	R	S	0	1	1	23	1258960
Megachile frugalis	4.72	P	C	R	S	0	1	1	19	3207520
Megachile lippiae	4.58	P	M	E	S	1	1	1	14	2083760
Megachile montivaga	4.58	M	C	R	S	1	1	0	23	5244100
Megachile onobrychidis	4.29	P	M	R	S	1	1	1	20	2598760
Megachile parallela	5.72	O	M	E	S	0	1	1	7	4265410
Megachile subnigra	5.29	O	M	R	S	1	1	0	24	915377
Osmia californica	5.15	O	C	R	S	1	1	0	24	1372730
Osmia clarescens	4.15	P	C	R	S	1	1	0	20	641647
Osmia coloradensis	4.43	O	C	R	S	1	1	0	24	1865130
Osmia gabrielis	5.01	P	C	R	S	1	1	0	20	315481
Osmia granulosa	3.58	P	C	R	S	1	1	0	20	3150490
Osmia grinnelli	5.01	O	C	R	S	1	1	0	24	353479
Osmia kincaidii	2.86	P	C	R	S	1	0	0	20	1335570
Osmia mixta	4.15	P	C	R	S	1	0	0	20	N/A
Osmia montana	4.58	O	C	R	S	1	1	0	24	1573240
Osmia nemoris	4.00	M	M	R	S	1	1	0	23	1539430
Osmia sp. 1	4.86	P	C	R	S	0	1	0	19	N/A
Protosmia rubifloris	2.15	P	C	R	S	1	1	0	20	976046
Stelis micheneri	1.86	C	M	R	S	1	0	0	13	18767
Stelis montana	3.00	C	C	R	S	1	1	0	16	2654490
Stelis cf. *hurdi*	4.29	C	G	R	S	0	1	0	12	N/A
Stelis trichopyga	2.72	C	M	R	S	0	1	0	12	N/A

						Flight season				
Species	ITL	Lecty	NL	NB	Soc.	E	M	L	FG	RS
Melittidae										
Hesperapis ilicifoliae	2.57	O	G	E	S	0	1	0	8	77370

Table 2-S2. Results of main analyses performed on the full dataset (i.e., with the inclusion of both bee specimens collected via aerial net and bowl traps) and on genus-level data (genus-level mean or modal averages were used for functional traits and range size). "Res" = study plots in natural reserves; "frag" = study plots in natural fragments. Results of these additional analyses are in qualitative agreement with results of the full dataset; in the few cases where these additional analyses did not attain statistical significance, the trends were in the same direction as those in the main analyses. The results of these additional analyses therefore support the conclusions drawn from our main analyses.

Analysis	Test statistic	*P*-value	Results
Genus-level analyses			
Plot-level *H* (2011)	$t_{5.94} = 4.14$	0.006	Res > frag
Plot-level H (2012)	$t_{11.64} = 5.62$	0.0001	Res > frag
Plot-level FDis (2011)	$t_{5.54} = 3.13$	0.023	Res > frag
Plot-level FDis (2012)	$t_{9.93} = 3.40$	0.007	Res > frag
Correlation between *H* and FDis	$t_{15.48} = 8.31$	< 0.0001	$R^2_m = 0.75$, $R^2_c = 0.91$
Taxonomic beta diversity (2011)	$F_{1,6} = 0.24$	0.72	No difference between res and frag
Taxonomic beta diversity (2012)	$F_{1,15} = 0.15$	0.72	No difference between res and frag
Functional beta diversity (2011)	$F_{1,6} = 0.04$	0.86	No difference between res and frag
Functional beta diversity (2012)	$F_{1,15} = 0.09$	0.76	No difference between res and frag
Taxonomic composition (2011)	$F_{1,6} = 2.47$	0.052	No difference between res and frag
Taxonomic composition (2012)	$F_{1,15} = 2.37$	0.023	Res ≠ frag in composition
Functional composition (2011)	$F_{1,6} = 3.89$	0.057	No difference between res and frag
Functional composition (2012)	$F_{1,15} = 4.11$	0.019	Res ≠ frag in composition
Range size (2011)	$t_{5.85} = 1.83$	0.12	No difference between res and frag
Range size (2012)	$t_{12.60} = 1.85$	0.087	No difference between res and frag
Full dataset analyses			
Plot-level *H* (2011)	$t_{5.73} = 4.42$	0.005	Res > frag
Plot-level H (2012)	$t_{14.15} = 5.41$	< 0.0001	Res > frag
Plot-level FDis (2011)	$t_{4.95} = 2.46$	0.058	No difference between res and frag
Plot-level FDis (2012)	$t_{11.08} = 3.14$	0.009	Res > frag
Correlation between *H* and FDis	$t_{23} = 7.88$	< 0.0001	$R^2_m = 0.72$, $R^2_c = 0.72$
Taxonomic beta diversity (2011)	$F_{1,6} = 0.28$	0.66	No difference between res and frag
Taxonomic beta diversity (2012)	$F_{1,15} = 0.07$	0.80	No difference between res and frag
Functional beta diversity (2011)	$F_{1,6} = 0.003$	0.94	No difference between res and frag
Functional beta diversity (2012)	$F_{1,15} = 0.17$	0.72	No difference between res and frag
Taxonomic composition (2011)	$F_{1,6} = 2.32$	0.030	Res ≠ frag in composition
Taxonomic composition (2012)	$F_{1,15} = 2.66$	0.003	Res ≠ frag in composition
Functional composition (2011)	$F_{1,6} = 5.09$	0.028	Res ≠ frag in composition
Functional composition (2012)	$F_{1,15} = 5.56$	0.004	Res ≠ frag in composition
Range size (2011)	$t_{5.87} = 1.97$	0.098	No difference between res and frag
Range size (2012)	$t_{12.56} = 3.10$	0.009	Res < frag

Table 2-S3. Results of analyses of taxonomic and functional beta diversity and assemblage composition, performed with the exclusion of indicator species associated with reserves. The exclusion of indicator species resulted in our finding no difference between study plots in reserves ("res") and fragments ("frag") with respect to the taxonomic and functional composition of their bee assemblages. These results therefore support our conclusion that the preferential extirpation of indicator species drives taxonomic and functional differentiation between reserves and fragments.

Analysis	Test statistic	*P*-value	Results
Taxonomic beta diversity (2011)	$F_{1,6} = 0.57$	0.51	No difference between res and frag
Taxonomic beta diversity (2012)	$F_{1,15} = 0.11$	0.75	No difference between res and frag
Functional beta diversity (2011)	$F_{1,6} = 0.18$	0.60	No difference between res and frag
Functional beta diversity (2012)	$F_{1,15} = 0.35$	0.58	No difference between res and frag
Taxonomic composition (2011)	$F_{1,6} = 2.16$	0.058	No difference between res and frag
Taxonomic composition (2012)	$F_{1,15} = 1.38$	0.20	No difference between res and frag
Functional composition (2011)	$F_{1,6} = 3.54$	0.091	No difference between res and frag
Functional composition (2012)	$F_{1,15} = 2.35$	0.088	No difference between res and frag

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CHAPTER 3: Plant-Pollinator Interaction Networks in Fragmented Habitats Retain Structural Robustness in Spite of Pollinator Diversity Loss

ABSTRACT

Research over the last two decades has uncovered general properties in the structure of plant-pollinator interaction networks that serve as useful metrics for examining the impacts of environmental change on pollination mutualisms. Surprisingly, despite the popular use of the network approach in research on plant-pollinator interactions, few studies have empirically examined how network metrics respond to one of the greatest threats to pollination mutualisms: pollinator diversity loss. Here, we documented 12 plant-pollinator interaction networks in study plots located in large natural reserves (n = 6) and small habitat fragments embedded in urban landscapes (n = 6) to test two hypotheses. First, we hypothesized that pollinator diversity loss modifies the structure of networks, which we predicted should exhibit reductions in nestedness and the number of links per species, as well as experience increases in niche overlap and generalization in areas with low pollinator diversity. Second, we hypothesized that network structure is influenced by the introduction of super-generalist honey bees, whose presence in our system, we predicted, should contribute positively to all four of the abovementioned network metrics. Contrary to our expectations, pollinator diversity loss was related to increased network nestedness and reduced network-level generalization within each habitat category, and had no impact on niche overlap and the number of links per species. On the other hand, honey bees influenced network properties in the manner we expected, contributing positively to network nestedness, niche overlap, the number of

links per species, and network-level generalization. Our findings suggest that changes in network structure as a result of reduced pollinator diversity caused networks to gain, rather than lose, structural robustness through enhanced nestedness, such that levels of pollinator diversity loss documented here are unlikely to impact the long-term functionality of plant-pollinator interaction networks in our system. More broadly, we demonstrated that changes in network structure as a result of species removals may deviate from theoretical predictions, thus calling to attention the need for more data regarding how networks are disassembled in empirical systems.

INTRODUCTION

Animal-mediated pollination of angiosperms represents a vital ecosystem function in terrestrial ecosystems (Kearns and Inouye 1997, Ashman et al. 2004, Ollerton et al. 2011). Thus, reported declines in pollinator abundance and diversity worldwide (Biesmeijer 2006, Winfree et al. 2009, Potts et al. 2010, Goulson et al. 2015) could harm the integrity of terrestrial ecosystems. For this reason, documenting how environmental change impacts the structure and function of plant-pollinator interactions has been identified as an important research priority (Mayer et al. 2011, Cardinale et al. 2012).

In the last two decades, the bipartite network approach (Memmott 1999) has become widely favored for examining interactions between communities of flower-visiting animals and plants. To construct plant-pollinator interaction networks, researchers document the frequency with which each pollinator species visits each plant species within a predefined area. The resulting topology of interaction patterns provides information regarding the manner in which species are connected to one another

(Strogatz 2001, Bersier et al. 2002), and the number of interactions documented between two species is often used as a surrogate for the strength of the relationship between the two putative mutualists with respect to pollination services or food provision (Vázquez et al. 2005, 2012).

While studies on the structure of plant-pollinator interaction networks provide no direct information on the fitness of organisms involved, general patterns in network structure across plant and pollinator communities (Olesen et al. 2007, Bascompte and Jordano 2007) have shed light on the function of these networks. For example, nestedness (Bascompte et al. 2003) and asymmetry (Vázquez and Aizen 2004, Bascompte et al. 2006), two properties common to most networks studied, result from the presence of groups of numerically abundant, ubiquitous generalist species that interact with large numbers of partner taxa. Having such generalized species at the "core" (Bascompte et al. 2003) of networks may cause the ecological function of these networks (i.e., pollination and food provision) to be robust to the loss of species (e.g., Memmott et al. 2004, Abramson et al. 2011) and habitat (Fortuna and Bascompte 2006). However, if network structure indeed predicts the resiliency of ecological relationships between plants and pollinators, then perturbations to network structure resulting from anthropogenic impacts may have strong consequences on ecological function (e.g., Aizen et al. 2012)

Given the link between network structure and ecological function, a number of studies have investigated how plant-pollinator interaction networks are impacted by different sources of anthropogenic impact such as habitat fragmentation (e.g., Spiesman and Inouye 2013), land use intensification (e.g., Marrero et al. 2014), grazing (Yoshihara et al. 2008), and biological invasions (Bartomeus et al. 2008). These studies reveal that

while anthropogenic impacts often reduce species richness in networks as expected, properties of networks do not always respond to the loss of species as theory would predict (e.g., Burkle and Knight 2012, Spiesman and Inouye 2013); such deviations from theoretical predictions call into question the robustness often attributed to plant-pollinator interaction networks (Santamaría et al. 2016). Thus, while general patterns have been uncovered regarding the overall structure of networks under relatively natural settings, empirical data are still needed to build consensus regarding how species loss impacts network structure and function (Bascompte and Stouffer 2009, Santamaría et al. 2016).

Here, we examine the structure of twelve plant-pollinator interaction networks in a species-rich ecosystem where we have previously documented a marked reduction in pollinator diversity associated with habitat fragmentation (see Chapters 1 and 2 of this dissertation). Our system is also numerically dominated by the non-native western honey bee (*Apis mellifera* L.), providing the opportunity to assess the role of this widespread generalist species in networks in its introduced range. Our data allow us to address two hypotheses: (1) pollinator diversity loss impacts the structural properties of networks so as to erode network robustness or functionality, and (2) network structure is influenced by the introduction of the numerically dominant, generalized honey bee.

Although there are now numerous metrics that describe different properties of networks (Dormann et al. 2009), our test of the first hypothesis focuses on four metrics that are often reported and whose relationships with ecological function are mechanistically well-understood: (1) nestedness, (2) niche overlap, (3) number of links per species, and (4) generalization. A network with a nested interaction structure is organized around a set of numerically abundant, generalized pollinator and plant taxa that are well connected

(Bascompte et al. 2003) and presumably provide a disproportionate amount of pollination services and food resources (Vázquez et al. 2012, Kleijn et al. 2015). Given previous findings that rare and specialized species, which tend to enhance nestedness (Bascompte et al. 2003), are more prone to local extinction in fragmented landscapes (Cane et al. 2006, Biesmeijer 2006, Aizen et al. 2012), we predict that pollinator diversity loss should decrease network nestedness.

Niche overlap measures the degree to which species in a trophic level (in this case, plants or pollinators) share patterns of interactions (Dormann et al. 2009). Low niche overlap may indicate effective resource partitioning among putative competitors or signify the presence of functionally diverse assemblages capable of supporting a high diversity of partners (Blüthgen and Klein 2011). Low niche overlap may also indicate a lack of functional redundancy, and therefore, a greater vulnerability of ecosystem function to the loss of species (Devoto et al. 2012). We predict that pollinator diversity loss should increase niche overlap as more specialized species are removed, leaving behind more generalized pollinator species that visit the same set of generalized plant species.

The number of links per species measures the degree to which possible interactions between plants and pollinators are realized (Sabatino et al. 2010) and serves as a metric of the robustness of ecosystem function. Previous research has shown that links tend to be lost at a faster rate than species when habitat area is reduced (Sabatino et al. 2010, Burkle and Knight 2012); therefore, we also predict that the number of links per species will decrease with pollinator diversity loss in our system.

Generalization refers to the degree to which plant and pollinator species interact with many, rather than few, partners. We expect pollinator diversity loss to increase network-level generalization via two distinct mechanisms. First, as discussed above, the preferential removal of specialists in typical scenarios of biodiversity loss should yield networks composed of more generalized species. Second, studies in both natural ecosystems (Brosi and Briggs 2013) and experimental mesocosms (Fründ et al. 2013) have shown that generalist pollinators may exhibit reduced selectivity when pollinator diversity is reduced, presumably because exploitative competition forces species to focus foraging efforts on only the food resources on which they forage with higher efficiency relative to their competitors (Pimm et al. 1985, Bolnick et al. 2010).

In our system, the non-native western honey bee reaches high abundances, largely due to the proliferation of feral, Africanized colonies (Kono and Kohn 2015). Generalists form the core of interaction networks (Bascompte et al. 2003), thus, the addition of an abundant super-generalist (Giannini et al. 2015, Geslin et al. 2017) should have a disproportionate impact on network architecture. Specifically, we predict that honey bees should contribute to enhanced network nestedness, greater number of links per species, increased niche overlap, and increased generalization.

Like many other studies taking place in fragmented or otherwise modified landscapes (Forup et al. 2007, Power and Stout 2011, Aizen et al. 2012, Spiesman and Inouye 2013, Moreira et al. 2015), our networks span a gradient of pollinator diversity. However, unlike most of these studies, we selected habitat fragments where plant communities have remained relatively intact, thus enabling us to study the effect of pollinator diversity loss in isolation from the effects of eroding entire networks.

Additionally, previous empirical research investigating the impacts of exotic species on plant-pollinator interactions have mostly focused on non-native plants (Carvalheiro et al. 2008, Bartomeus et al. 2008, 2010, Gibson et al. 2012, Ferrero et al. 2013, Larson et al. 2016) rather than pollinators (Kato and Kawakita 2004, Abe et al. 2011, Giannini et al. 2015). Thus, our study also provides valuable data regarding the degree to which network structure responds to pollinator diversity loss in a system dominated by a non-native generalist (Traveset and Richardson 2014).

MATERIALS AND METHODS

Study System: Data collection occurred in the coastal sage scrub (CSS) ecosystems of San Diego County, CA. CSS, and the American Southwest in general, is a global diversity hotspot for native bees (Michener 1979), which are among the most important pollinators in temperate ecosystems (Kearns and Inouye 1997). In San Diego, CSS also supports a rich assemblage of largely insect-pollinated, endemic plants (Jensen et al. 1993). Less than 15% of original coastal sage scrub habitat currently remains (Jensen et al. 1993), and the remnants of this unique ecosystem consist largely of habitat fragments embedded in altered landscapes (i.e., urban and agricultural areas). This system of discrete, isolated scrub fragments has been used to study the effects of habitat fragmentation on a diversity of animal taxa (Bolger et al. 1991, 2008, Soulé et al. 1992, Suarez et al. 1998).

In 2015 and 2016, we set up 1-ha study plots in large natural reserves ($n = 6$; internal area >> 500 ha) and small fragments of CSS habitat ($n = 6$; internal area < 60 ha). Locations of the study plots are reported in Table 3-S1. Previous sampling at these sites

revealed that fragments supported native bee assemblages with reduced taxonomic and functional diversity compared to those found in reserves (see Chapters 1 and 2 of this dissertation). Since these study plots were originally chosen in an attempt to investigate the effects of habitat fragmentation *per se* (i.e., independent of other consequences of urbanization such as landscape simplification), reserves and fragments harbored plant assemblages similarly representative of intact coastal sage scrub flora (Appendix 3-1).

Field data collection: Between March and July of 2015 and 2016, we documented putative pollinators as they visited flowers of a set of focal plant species naturally growing in our study plots. Here, we defined putative pollinators as flying insects belonging to the orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera (additionally, one humming bird was recorded in our sampling). Since plant species in our system often exhibit clumped distributions, we performed pollinator surveys using a timed observation method (Gibson et al. 2011). Compared to the frequently used transect method, the timed observation method grants superior ability to investigate network properties of rarer plant species (Gibson et al. 2011), which comprise the majority of plant diversity at our sites. During each survey, we observed a single patch of plants for ca. 60 s, counting all putative pollinators already present on the patch as well as pollinators that arrived at the patch; after this time we moved on to the next patch. Patches ranged in size from a portion of inflorescences of one plant individual for large shrubs (e.g., *Malosma laurina* (Nutt.) Abrams) to several hundred individuals for annual forbs (e.g., *Deinandra fasciculata* (DC.) Greene), and was determined by our ability to keep track of putative pollinators in our field of view. Pollinators that are identifiable to species in the field were counted (e.g., honey bees, large syrphid flies and butterflies); all

others were collected, pinned, and individually identified in the laboratory. We documented each pollinator individual that appeared to contact reproductive parts of flowers (i.e., the anther or stigma) as a single interaction, and took care not to count the same pollinator individual multiple times in the same patch. We collected roughly one-third of all non-honey bee pollinator individuals we encountered (33.8% in 2015, 34.2% in 2016); and in the vast majority of cases, pollinator individuals that were counted but not collected were unambiguously matched with specimens collected from the same site on the same survey day. Because our survey protocol did not consistently allow detection of minute insects under field conditions, we only documented pollinators ≥ 2 mm, which represents the minimum size at which we can reliably spot insects.

In 2015, we equally divided 120 min of survey time per study plot among all focal plant species in which more than ca. 5% of the flowers were in bloom. We limited survey time to 20 min per plant species when fewer than six plant species were blooming in a given plot. In 2016, we allocated 15 min to each plant species irrespective of how many plant species were in bloom at a given study plot. While our sampling methods prioritized standardizing sampling effort among study plots in 2015 and across all plant species in 2016, all plant species at a given study plot received the same sampling effort on any given day. Additionally, study plots received community-level plant-pollinator surveys for the same number of days in each year. Given that we always allocated the same amount of time to each plant species within each plot in a given visit, the structure of networks and the relative interaction strengths of plants and pollinators documented at a given plot should be roughly comparable between years. The protocols and sampling effort we employed are comparable to those in other studies that have documented floral

visitation networks in detail (Alarcón et al. 2008, Gibson et al. 2011, Spiesman and Inouye 2013).

Properties of plant-pollinator interaction networks: Using data from the floral visitation surveys, we constructed plant-pollinator interaction networks (Memmott 1999) where each plant and pollinator species is represented by a node, and the number of observations recorded between each unique pair of plant and pollinator taxa serves as a proxy for the strength of ecological relationships between the two partners (Vázquez et al. 2005, 2012). We pooled all observations from each study plot in each study year together into a single network, resulting in 24 total networks across the two study years. Then, we used package *bipartite* (Dormann et al. 2009) in program R (R Development Core Team 2015) to calculate network metrics to test predictions regarding the effects of pollinator diversity loss and exotic pollinator introduction on network structure.

To estimate nestedness, we calculated weighted nestedness based on overlap and decreasing fill (NODF, Almeida-Neto and Ulrich 2011), a metric that identifies truly nested patterns more consistently and precisely than competing metrics (Almeida-Neto and Ulrich 2011). To examine the degree of niche overlap, we calculated the weighted Horn-Morisita similarity index of interaction patterns among species as recommended by (Dormann et al. 2009). Given that our focus was to examine the relationship between pollinator diversity and network structure, we report niche overlap for pollinators only. The number of links per species equals the number of distinct links divided by the sum of pollinator and plant species richness. Lastly, while not a traditionally considered network statistic *per se*, we also calculated the number of plot-level singleton species (i.e., species represented by a single individual), as well as the proportion of species at each plot

consisting of singletons, to test the hypothesis that rare species are preferentially extirpated when species richness is reduced.

To test the hypothesis that pollinator diversity loss leads to more generalized networks, we calculated H_2', a network-level metric of interaction selectivity integrated across both pollinators and plants (Blüthgen et al. 2006). H_2' measures the degree to which plant and pollinator species in a network interact with specific sets of partners, as opposed to distributing their interactions among possible partners based on each partner's relative abundance (Blüthgen et al. 2006). As such, H_2' is relatively robust to variation in the number of species and individuals sampled, facilitating direct comparisons between networks (Blüthgen et al. 2006).

Statistical analyses: We constructed multiple linear regression models to examine how pollinator diversity loss influences network properties, where each measured network statistic is a dependent variable. Independent variables were selected among habitat category (reserves versus fragments) and the species richness of pollinators and plants recorded in each network. To control for among-site variation in the number of interactions documented, pollinator species richness was rarefied to the lowest site-level number of interactions documented each year (n = 1,000 subsamples), excluding honey bees. Plant species richness was included as a candidate covariate to control for the effects of increased network size on network structure (Blüthgen 2010). We constructed all combinations of the three independent variables (without interactions) using R package *glmulti* (Calcagno et al. 2010), and chose the model with the lowest corrected Akaike's Information Criterion (AICc) score. When multiple models achieved similar fit (ΔAICc < 2), we chose the minimum sufficient model, or the model that was

selected as a top model in both study years. When no model achieved superior AICc scores compared to the null (intercept-only) model, we report the results of the model with only rarefied pollinator richness included. Lastly, habitat category and rarefied pollinator richness are related to each other because the former was chosen to generate a gradient in the latter. Fragment plots had, on average, a 22.5% reduction in rarefied pollinator richness relative to reserves across the two study years (two-sample *t*-test $t_{9.91}$ = 2.11, P = 0.061 in 2015; $t_{7.92}$ = 3.08, P = 0.015 in 2016). However, given the low variance inflation factor of these two variables (VIF = 1.32 in 2015; VIF = 1.79 in 2016), their relationship does not appear sufficiently strong for multicollinearity to affect our conclusions in cases where the best model includes both independent variables. In all analyses, data from the two study years were analyzed separately.

To test the hypothesis that non-native honey bees modify the structure of plant-pollinator interaction network structure in our system, we calculated all aforementioned network-level statistics after excluding honey bees from our dataset. Then, we compared honey bee-present versus honey bee-absent datasets with respect to each statistic using paired *t*-tests, combining networks from both reserves and fragments. We also calculated the proportion of all interactions consisting of honey bees at each site, and used the model selection process described above (with habitat category, pollinator richness, and plant richness as independent variables) to examine whether the relative abundance of honey bees varies across the gradient of pollinator diversity loss. Data from the two study years were analyzed separately.

Lastly, in addition to examining the impacts of pollinator diversity loss and introduced pollinators on the structural properties of network, we performed a correlation

analysis to examine the degree to which each network statistic varied across the two years of our study. The structure of pollination networks is known to vary from year to year in a given locality (Alarcón et al. 2008); our use of slightly different sampling methods in the two study years may also contribute to differences in the data. This analysis thus serves to identify network statistics that are robust to inter-annual variation in sampling methodology and population dynamics of plants and pollinators.

RESULTS

Across two years of sampling, we documented 35,481 individuals of at least 400 pollinator taxa (including unidentified morphotypes that may belong to multiple species) visiting 57 plant species (Hung et al. 2017). Of these pollinators, 26,492 were honey bees, and 5,551 were native bees belonging to 163 species in 6 families. As with many other systems, rare species contributed significantly to our total pollinator richness (Williams et al. 2001). We documented 86 singletons in 2015 and 105 singletons in 2016; of these, 123 singleton species were documented only once across the two study years. Natural reserves contained more singleton species in both years (Fig. 3-1A); whereas the proportion of singleton species in the species pool was unrelated to pollinator richness in either year (pollinator richness model: $F_{1,10} = 1.12$, $P = 0.31$ in 2015; $F_{1,10} = 0.26$, $P = 0.62$ in 2016).

Network structure: Counter to our prediction, network nestedness (weighted NODF) was higher in fragments than in reserves (Fig. 3-1B), and niche overlap of pollinators was unrelated to either habitat category or pollinator richness, being only related to plant richness in both years ($F_{1,10} = 7.07$, $P = 0.024$ in 2015; $F_{1,10} = 10.87$, $P =$

0.008 in 2016). Similarly, the number of links per species responded only to plant richness in 2015 ($F_{1,10}$ = 12.91, P = 0.005), and to no measured independent variable in 2016 (pollinator richness model: $F_{1,10}$ = 0.19, P = 0.67). Consistent with our predictions, network-level interaction selectivity (H_2') was higher in reserves than in fragments (Fig. 3-1C-D; t = 2.70, P = 0.025 in 2015; t = 2.83, P = 0.020 in 2016); however, counter to our prediction, selectivity was negatively related to pollinator richness (Fig. 3-1C-D, t = 2.60, P = 0.029 in 2015; t = 2.75, P = 0.023 in 2016).

Role of honey bees in networks: As predicted, exclusion of honey bees from networks reduced nestedness (Fig. 3-2A), niche overlap (Fig. 3-2B), and the number of links per species (Fig. 3-2C). Also as predicted, excluding honey bees yielded networks with increased selectivity (Fig. 3-2D). While excluding honey bees from analyses resulted in profoundly different network properties (statistically significant using two-sample t-tests) in the majority of cases, pollinator niche overlap changed only slightly, with a difference not significant when analyzed using a two-sample (rather than paired) t-tests ($t_{21.99}$ = 0.25, P = 0.80 in 2015; $t_{21.98}$ = 0.23, P = 0.82 in 2016). The proportion of total interactions contributed by honey bees was both higher in fragments in both years (Fig. 3-2E-F, t = 3.08, P = 0.13 in 2015; t = 5.12, P < 0.001 in 2016), and additionally, positively related to pollinator species richness in 2016 (Fig. 3-2F, t = 4.92, P < 0.001).

Inter-annual variation in network properties: While network properties are generally positively correlated across our two study years, the strength of the correlations varies considerably among metrics (Fig. 3-3). Pollinator richness, nestedness (weighted NODF), pollinator niche overlap, singleton species richness, the proportion of total documented interactions attributed to honey bees were all metrics that exhibited

significant correlations between the two study years, while correlations were not statistically significant at the $\alpha = 0.05$ level for the other three metrics investigated (Fig. 3-3).

DISCUSSION

We demonstrate that habitat fragmentation and its associated reductions in site-level pollinator diversity alter the structure of plant-pollinator interaction networks. However, these changes ran counter to our expectations in several respects, and suggest that the structure and function of networks in our system may be more or less robust to the level of pollinator diversity loss documented here. The robustness of network structure to species loss resulted in part from the high numerical dominance of the non-native honey bee, a super-generalist that contributed the majority of floral visits in every study plot. Despite the finding that our networks exhibited notable inter-annual variation with respect to many of the statistics we measured, the majority of our analyses yielded qualitatively similar conclusions in the two study years, bolstering our confidence that our findings resulted from real biological phenomena rather than chance.

While we predicted that networks in fragments (where pollinator richness is lower) should exhibit reduced nestedness relative to reserves, we found the opposite pattern. This surprising finding runs counter to other studies that have found nestedness to be positively related to pollinator species richness (Burkle and Knight 2012, Spiesman and Inouye 2013, Moreira et al. 2015). Two mechanisms could lead to an increase in nestedness: the addition of species, individuals, or links that contribute to nested patterns, or the removal of those that reduce nestedness (Bascompte et al. 2003). In our system,

there is no evidence that fragments experienced relative gains in species whose interaction patterns would contribute to nestedness, such as specialists that visit the most generalized plant species or generalist species that visit the majority of plant species. Thus, the increased nestedness in fragment networks is likely attributable to their experiencing a combination of removal of species whose interaction patterns do not conform to nestedness (e.g., those that visit plants not preferred by more generalized species). Whatever the mechanism, higher nestedness in fragment networks may buffer the structure and function of these networks from further perturbation (Bascompte et al. 2003), especially if drivers of pollinator species loss tend to extirpate specialists or rare species first (Fortuna and Bascompte 2006, Burgos et al. 2007).

The increased nestedness in fragment networks may be related to another unexpected finding, that pollinator niche overlap did not respond to pollinator diversity loss. Niche overlap measures the overall similarity of interaction patterns among pollinators in a network (Dormann et al. 2009). In this theoretical framework, extirpating species whose interaction patterns are similar to those of extant species will tend to decrease niche overlap, while extirpating species that occupy uncommon niches (e.g., visit plant species that few other species visit) will increase niche overlap (Dormann et al. 2009). Thus, for niche overlap to remain relatively unaffected by pollinator species loss, the extirpated species must have intermediate levels of overlap with other pollinators. Pollinator species with an intermediate number of plant partners that are not proper subsets of more generalized pollinator species may fall into this category of intermediate niche overlap; the extirpation of these species, as discussed above, would also increase network nestedness.

The results of our analysis of pollinator niche overlap deviate from two different, but ecologically reasonable, expectations regarding patterns of species loss in modified habitats. The first expectation is that highly connected generalists persist when more specialized species are extirpated (Fortuna and Bascompte 2006), thereby increasing niche overlap. The second expectation is that habitat alteration reduces the functional redundancy of biological communities (Flynn et al. 2009, Laliberté et al. 2010), thereby reducing niche overlap. Given that our results deviate from these expectations, our findings represent an interesting ecological phenomenon worthy of further investigation. A number of other studies have described the degree of niche overlap in plant-pollinator interaction networks (Power and Stout 2011, Traveset et al. 2013, Cusser and Goodell 2013, Giannini et al. 2015), but few have empirically investigated this metric in the context of pollinator species loss (Marrero et al. 2017), and thus general patterns relating niche overlap to pollinator diversity remain to be uncovered.

Our third expectation regarding the impact of pollinator species loss on network structure—that links will be lost at faster rates than species—was also not supported by our data. While the minimum number of links in a network could be as low as half of the number of species present (i.e., in a networks where each species only interacts with a single partner), the generalized and asymmetric structure of most networks (Vázquez and Aizen 2004) generally yields a larger number of links than species (Bascompte et al. 2003). Empirical studies have also found that links may increase (and decrease) at a faster rate compared to the accumulation (and loss) of pollinator species, both due to sampling effects (Burkle and Knight 2012) and to the underlying biology of interaction networks (Sabatino et al. 2010). In our case, the number of links per species is indeed

higher than the number of species present, but not as high as reported in other studies (Sabatino et al. 2010, Burkle and Knight 2012). The relatively low number of links per species in our system may be driven by the large number of rare species (Fig. 3-3F-G) whose addition to the network adds only one additional link each, thereby shifting the average number of links per species closer to one. From a conservation standpoint, our finding that the number of links per species was not altered by species loss represents another line of evidence that network structure in our system is robust to habitat alteration, at least when plant assemblages remain intact.

As with our analyses of network structure, our analyses of pollinator generalization received yielded unexpected results. Network-level interaction selectivity (H_2') was indeed higher in reserves than in fragments, but it was negatively, not positively, related to pollinator diversity. Since reduced selectivity by pollinators may negatively impact the reproductive success of plants they visit via reducing conspecific pollen transfer (Fründ et al. 2013, Brosi and Briggs 2013), plants in our fragment plots may suffer reduced reproductive success as a result of reduced H_2' relative to reserves. On the other hand, enhanced network-level interaction selectivity (and potentially, enhanced conspecific pollen transfer as a result) in species-poor networks may, to some degree, buffer the erosion of pollination services in networks that have suffered the greatest extent of pollinator diversity loss. Interestingly, Burkle and Knight (2012) found a similar pattern in which habitat size was positively related to species richness, but negatively related to H_2'; the authors attributed their finding to an increase in the selectivity of numerically abundant generalist pollinators in smaller habitats. In our system, there was no evidence of a negative relationship between the selectivity of

numerically abundant generalist pollinators and pollinator species richness or habitat size (Appendix 3-2). However, it is interesting to note that patterns in H_2' resembled patterns in honey bee proportional abundance, which likewise exhibited opposing responses to habitat fragmentation and pollinator diversity loss. While elucidating the mechanisms underlying these intriguing findings is beyond the scope of this study, we can infer from these results that habitat fragmentation impacts plant-pollinator mutualisms above and beyond the effects of removing pollinator species. Habitat modification has also been documented to strongly alter networks of interactions among organisms independently of impacts on species richness (Tylianakis et al. 2007); these findings underscore the complexity of ecological interactions and highlight the need to take into account the natural history of organisms when predicting how the structure and function of biological communities may respond to anthropogenic impacts.

Unlike our predictions regarding network structure, our hypothesis regarding the role of honey bees in networks was wholly supported by our analyses (Fig. 3-2). Our results corroborate the findings of other studies (Moreira et al. 2015, Giannini et al. 2015, Geslin et al. 2017) that the super-generalist honey bee behaves as a ubiquitous and highly connected network "core" species (Bascompte et al. 2003, Aizen et al. 2012) that enhances network nestedness, increases overall network generalization, and contributes a disproportionate number of links. As such, it also likely performs the majority of pollination services in our system (Vázquez et al. 2012, Kleijn et al. 2015), at least to the plant species it visits frequently and effectively (Ballantyne et al. 2015). While we found that the honey bee is by far the most numerically dominant pollinator in our system, it is also important to note that the exclusion of honey bees from our analyses did not

qualitatively alter our findings regarding the impacts of habitat fragmentation and pollinator diversity loss on properties of networks (Appendix 3-2, Table 3-S2). Thus, our conclusions do not appear to be driven by the finding that honey bee abundance varied with pollinator richness and differed between habitat categories.

While comparisons of network metrics with *versus* without the inclusion of honey bees yielded results consistent with theoretical predictions regarding the loss of highly connected generalists (Burgos et al. 2007), this analysis does not necessarily provide insight into the consequences of physically removing honey bees from the ecosystem (as in Wenner and Thorp 1994). In real-world systems, the presence or absence of a numerically dominant pollinator species can elicit profound behavioral (Brosi and Briggs 2013) and numerical (Thomson 2016) responses in other pollinator taxa, and links among plants and pollinators may shift in response to species removals so as to maintain network robustness (Kaiser-Bunbury et al. 2010, Valdovinos et al. 2013). However, this analysis does shed light on the honey bee's current role in structuring networks and its potential to impact the fitness (Cane and Tepedino 2016) and evolution (Mu et al. 2014) of co-occurring plants and pollinators. While numerically abundant generalists are thought to be relatively resistant to extirpation (Fortuna and Bascompte 2006, but see Abramson et al. 2011), there is at least one report of precipitous declines in unmanaged honey bee populations in the past (Kraus and Page 1995). Given that factors related to increased mortality in honey bee populations (vanEngelsdorp et al. 2009) remain pervasive in many ecosystems worldwide (Goulson et al. 2015), it is essential for conservation efforts to secure the structure and function of plant-pollinator interaction networks irrespective of current contributions by honey bees.

As with other studies, we found notable year-to-year variation in the structural properties of our networks (Alarcón et al. 2008), such that data from the two years were uncorrelated with respect to three of the metrics we calculated (Fig. 3-3). However, perhaps more surprising is our finding that nestedness (weighted NODF), the proportion of interactions attributed to honey bees, and the number of singleton species in each network were largely consistent across the two years of sampling, despite the fact that the two years differed markedly in their temperature profile and the timing and quantity of precipitation (Western Regional Climate Center 2017). Pollinator assemblages are known to be highly variable from year to year (Williams et al. 2001), as well as exhibit time lags in their response to environmental conditions (Potts et al. 2003). The network metrics that exhibit high consistency from year to year in spite of fluctuations in plant and pollinator diversity, distribution, and phenology (Rafferty et al. 2015) may thus provide insight into how patterns of interactions between mutualists structure communities at a locality over longer timescales. Additionally, metrics that are robust to inter-annual variation in plant and pollinator assemblages at a locality may be candidate metrics that enable quantitative comparisons between networks with different spatial and temporal origins (Gibson et al. 2011).

CONCLUSIONS

We discovered that the structure of plant-pollinator networks in our system remained robust to the loss of pollinator species richness. In fact, networks appeared to gain resistance against further loss of structural integrity (and presumably, ecological function) as pollinator species are lost due to habitat fragmentation. Our finding of

multiple counterexamples to the predictions of prevailing theory also underscores the need for more research examining plant-pollinator interaction network structure and function in species-rich systems experiencing pollinator species loss and integration of novel pollinators. Lastly, while networks provide an excellent glimpse into patterns of interactions between plants and pollinators, more research is needed to mechanistically map the relationship between the myriad of network statistics available today (Dormann et al. 2009) to empirical measures of plant and pollinator fitness and population dynamics.

ACKNOWLEDGMENTS

We gratefully acknowledge the taxonomic assistance of T. Griswold, M. Orr, Z. Portman, and D. Yanega, as well as field and laboratory assistance of V. Apaldetti, P. Arroyo, D. Barros, A. Dipaola, C. Lin, B. Nguyen, K. Powell, L. Rowe, C. Shough, S. Sandoval, Y. Wang, and B. White. We also gratefully acknowledge J. Kohn, L. Levin, J. Minster, and J. Nieh for providing constructive feedback on our manuscript. This work was performed in scrub habitats managed by the University of California Reserve System (Elliott Chaparral Reserve), Open Space Parks in San Diego County (cities of La Mesa and San Diego), and the Otay-Sweetwater Unit of the San Diego National Wildlife Refuge. This work was funded by the NSF Doctoral Dissertation Improvement Grant DEB-1501566; the Mildred E. Mathias Graduate Student Research Grant and the Institute for the Study of Ecological & Evolutionary Climate Impacts Graduate Fellowship from the University of California Natural Reserve System; the Frontiers of Innovation Scholar Fellowship, the Academic Senate Grant, and the McElroy Fellowship from the University

of California, San Diego; the Sea and Sage Audubon Society Bloom-Hays Ecological Research Grant; and the California Native Plants Society Educational Grant.

Chapter 3, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Cen, Henry J.; Lee, Adrienne; Holway, David A. The dissertation author was the primary investigator and author of this manuscript.

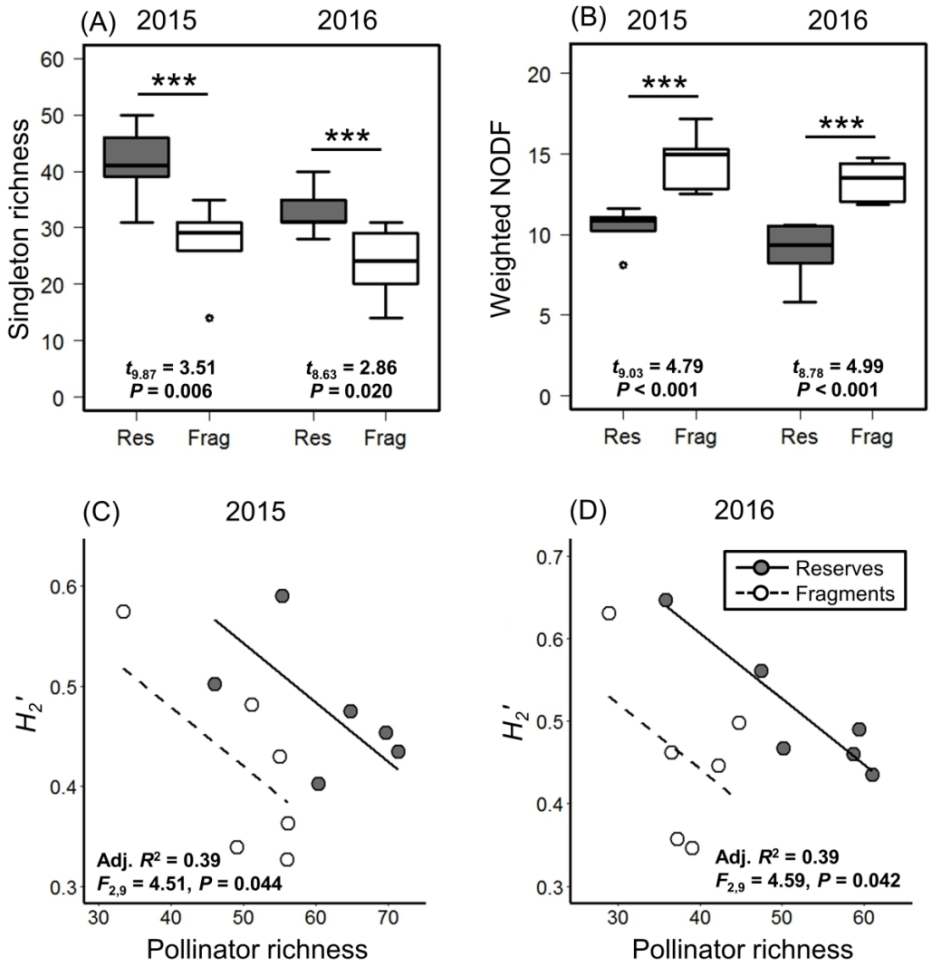

Figure 3-1. Plant-pollinator interaction network metrics that responded to habitat fragmentation, pollinator diversity loss, or both. Panels show (A) the raw species richness of singleton pollinators at the plot level (species that appear as singletons across multiple networks are included multiple times), (B) weighted nestedness based on overlap and decreasing fill (weighted NODF), a measure of network nestedness where higher values indicate structures closer to perfect nestedness, and (C-D) network-level interaction selectivity (H_2'), a measure of the network-level generalization of interactions where higher values indicate higher selectivity (and thus lower generalization). Pollinator richness was rarefied to match the lowest number of interactions documented in a network in each of the two study years. Boxes show central 50% of data and median; whiskers show quantiles ± 1.5 × interquartile range, or most extreme values of data, whichever is closest to median. * P < 0.05; ** P < 0.01; ***P < 0.005.

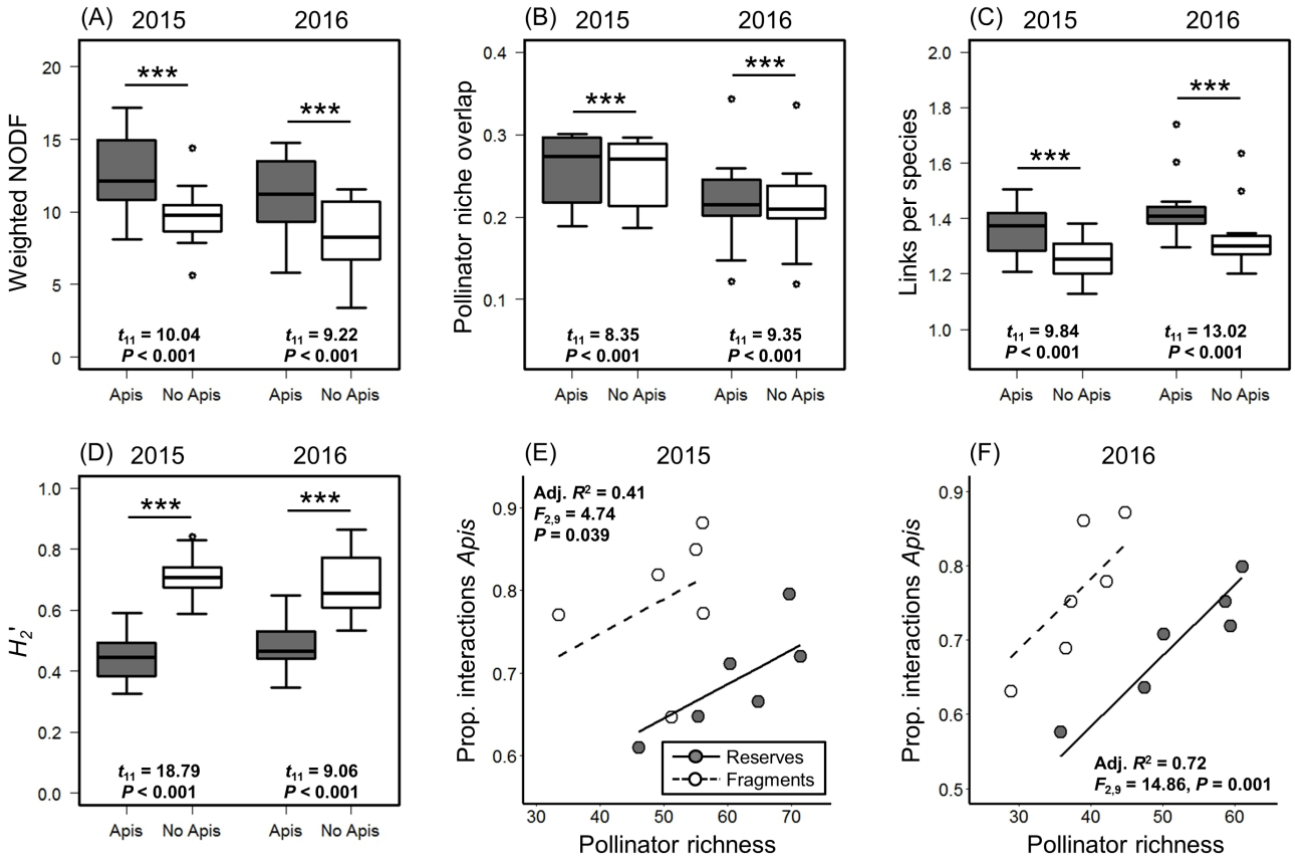

Figure 3-2. The role of honey bees in plant-pollinator interaction networks. Plots (A-D) show results of paired *t*-tests comparing network statistics between datasets that included *versus* excluded honey bees, demonstrating the contribution of honey bees to plant-pollinator interaction network structure. Boxes, asterisks, and abbreviations of independent variables are as in Fig. 3-1. Plots (E-F) show the relationship between the proportion of interactions in each network attributed to honey bees and both habitat category (reserves *versus* fragments) and rarefied pollinator species richness in networks.

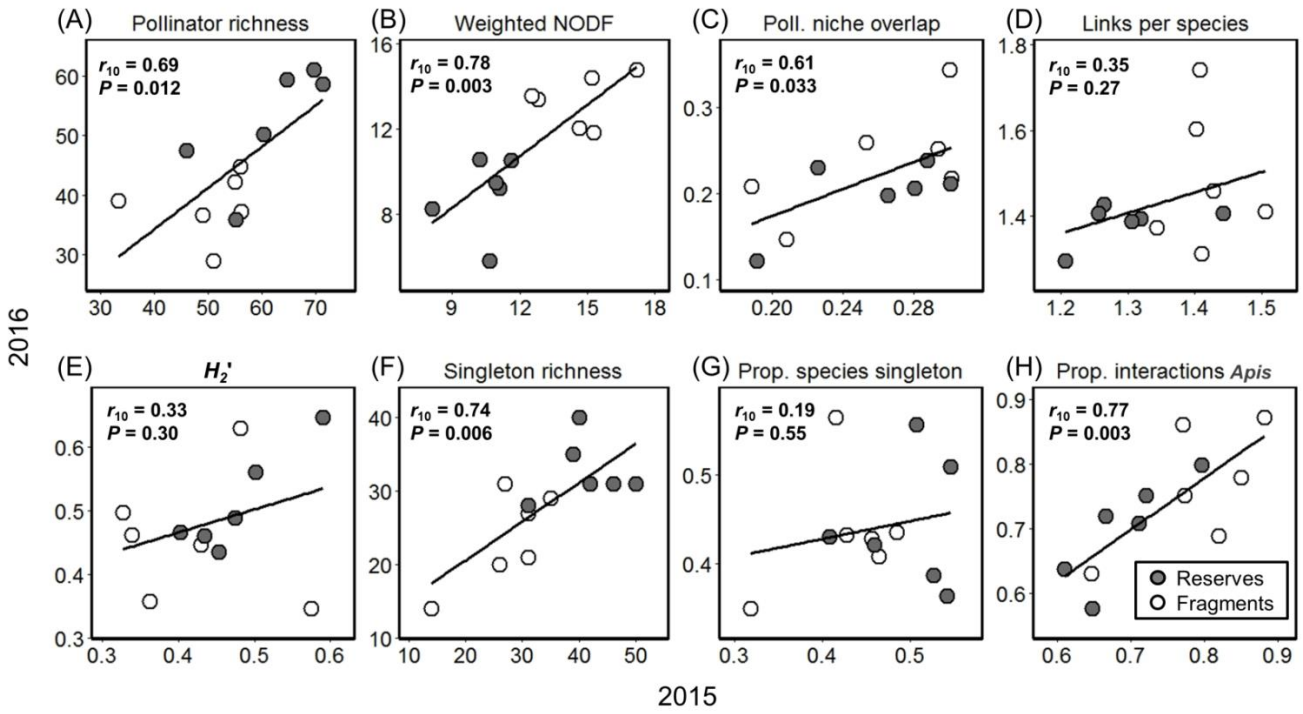

Figure 3-3. Year-to-year variation in the network statistics examined in our study. Abbreviations of independent variables are as in Fig. 3-1.

APPENDIX 3-1: Description and explanation of study sites.

Plots used in this study were located in the same system of habitat fragments and natural reserves surveyed in 2011 and 2012 (see Chapters 1 and 2 of this dissertation). Coordinates of study plots are reported in Table 3-S1. To verify that structural differences between networks in reserves and fragments are attributable to pollinator diversity loss rather than systematic differences in the composition of plant assemblages, we compared reserve and fragment plots with respect to the species richness of insect-pollinated plants and the abundance of perennial, insect-pollinated shrubs. We also compared the composition of insect-pollinated plant assemblages in reserves and fragments by performing permutational multivariate ANOVAs (PERMANOVAs) on plant presence-absence data, as in Chapter 1 of this dissertation (see Appendix 1-1).

Plant species richness did not differ between reserves and fragments in 2015 (two-sample *t*-test $t_{6.62} = 2.04$, $P > 0.05$) or 2016 ($t_{9.98} = 0.65$, $P > 0.05$). Similarly, the abundance of perennial, insect-pollinated shrubs did not differ between reserves and fragments ($t_{6.03} = 0.27$, $P > 0.05$; comparisons were based on data from 2016, but data from 2015 were similar). Reserves and fragments also did not differ with respect to plant assemblage composition in 2015 (PERMANOVA $F_{1,11} = 1.06$, $P > 0.05$) or 2016 ($F_{1,11} = 0.59$, $P > 0.05$).

Table 3-S1. List of utilized study plots in coastal sage scrub reserves and habitat fragments.

Plot	Frag / Res	Latitude	Longitude	Internal area (ha)
ECR4	Reserve	32.893	-117.092	> 500
ECR5	Reserve	32.900	-117.075	> 500
MTE3	Reserve	32.822	-117.076	> 500
MTE4	Reserve	32.835	-117.075	> 500
MTS1A	Fragment	32.792	-117.061	2.72
MTS2	Fragment	32.856	-117.188	12.89
MTS6	Fragment	32.722	-117.119	52.79
SWEA	Reserve	32.732	-116.956	> 500
SWI4	Reserve	32.727	-116.940	> 500
SWS1	Fragment	32.75	-117.032	46.55
SWS10	Fragment	32.786	-116.989	6.23
SWS3	Fragment	32.72	-117.078	28.06

APPENDIX 3-2: Supplemental analyses.

In order to examine whether or not the behavior of generalist pollinators varied with habitat category or pollinator richness (see Burkle and Knight 2012), we calculated species specificity (Poisot et al. 2012) on two groups of generalist pollinators. The first group consists of the honey bee, a super-generalist pollinator that contributed the majority of interactions we documented (see Results). The second group consists of the top 10 generalist pollinator species observed each year (see also Burkle and Knight 2012), defined as the 10 pollinator species for which we documented the highest numbers of individuals summed across all sites, among all pollinator species that were observed visiting plants from at least three different families. Species specificity is calculated as the coefficient of variation of the interactions between a species and all of its partners (Poisot et al. 2012), and enables us to examine, at the level of individual pollinator taxa, how pollinator diversity loss modifies the foraging choices of generalists. We constructed multiple linear regression models to examine the relationship between species specificity and habitat category (reserves *versus* fragments), rarefied pollinator species richness, and / or plant species richness; and selected the best model among all possible candidates using the procedure used in analyzing our four focal network metrics (see Materials and Methods). For this analysis, we averaged species specificity scores across all top 10 generalist pollinator species at each plot in each study year.

Additionally, in order to examine whether or not the contribution of honey bees to network structure (Fig. 3-2) modifies the extent to which network structure is impacted by pollinator diversity loss, we repeated all of the analyses pertaining to Hypothesis 1 (see Introduction and Methods) using data for which we excluded honey bees from calculations of each of the four focal network metrics (weighted NODF, pollinator niche overlap, the number of links per species, and H_2').

We found that species specificity of honey bees was related only to plant species richness in both 2015 ($F_{1,10}$ = 8.93, P = 0.013) and 2016 ($F_{1,10}$ = 6.11, P = 0.033). Species specificity of the top 10 pollinators was not related to habitat category or pollinator richness in either year (pollinator richness model: $F_{1,10}$ = 0.67, P = 0.43 in 2015; $F_{1,10}$ = 1.05, P = 0.33 in 2016). Additionally, we found that results of analyses of network metrics with the exclusion of honey bee data did not qualitatively differ from those of the full dataset (Table 3-S2). Thus, even though honey bee abundance varied with pollinator richness and differed between habitat categories (Fig. 3-2), their contributions to network properties (Fig. 3-2) did not qualitatively alter the impact of pollinator diversity loss on network structure.

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Table 3-S2. Re-analyses examining the impact of pollinator diversity loss on structural properties of plant-pollinator interaction networks, with honey bees excluded from the dataset. As in our main analyses, models reported below were selected from among candidate multiple linear regression models that included all possible combinations of three independent variables: habitat category (reserves *versus* fragments), rarefied pollinator species richness, or plant species richness, selected based on corrected AIC scores. In cases where no model achieved superior AICc scores compared to the null (intercept-only) model, we report the results of the model with only rarefied pollinator richness included.

Year	Model / Variable	Estimate	Test stat.	*P* value
2015	Weighted NODF ~ habitat category	Adj. $R^2 = 0.47$	$F_{1,10} = 10.65$	0.009
	Habitat category (res = 0, frag = 1)	$\beta = 2.95$	$t = 3.26$	0.009
	Pollinator niche overlap ~ plant richness	Adj. $R^2 = 0.36$	$F_{1,10} = 7.09$	0.024
	Plant richness	$\beta = -0.011$	$t = 2.66$	0.024
	# links per species ~ plant richness	Adj. $R^2 = 0.49$	$F_{1,10} = 11.65$	0.007
	Plant richness	$\beta = 0.022$	$t = 3.41$	0.007
	$H2'$ ~ habitat category + pollinator richness	Adj. $R^2 = 0.48$	$F_{2,9} = 5.98$	0.022
	Habitat category (res = 0, frag = 1)	$\beta = -0.12$	$t = 3.29$	0.009
	Pollinator richness	$\beta = -0.005$	$t = 2.71$	0.024
2016	Weighted NODF ~ habitat category	Adj. $R^2 = 0.35$	$F_{1,10} = 6.96$	0.025
	Habitat category (res = 0, frag = 1)	$\beta = 2.93$	$t = 2.63$	0.025
	Pollinator niche overlap ~ plant richness	Adj. $R^2 = 0.46$	$F_{1,10} = 10.42$	0.009
	Plant richness	$\beta = -0.010$	$t = 3.24$	0.009
	# links per species ~ pollinator richness	Adj. $R^2 = 0$	$F_{1,10} = 0.08$	0.78
	Pollinator richness	$\beta = -0.001$	$t = 0.28$	0.78
	H_2' ~ habitat category + pollinator richness	Adj. $R^2 = 0.57$	$F_{2,9} = 8.28$	0.009
	Habitat category (res = 0, frag = 1)	$\beta = -0.19$	$t = 3.17$	0.011
	Pollinator richness	$\beta = -0.012$	$t = 4.04$	0.003

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CHAPTER 4: The Worldwide Importance of Honey Bees as Pollinators in Natural Habitats

ABSTRACT

Honey bees are the single most important pollinator species for crops, but their importance as pollinators in natural plant communities remains controversial. Here we use a meta-analysis of 80 plant-pollinator network studies to assess the frequency of honey bee floral visitation in natural habitats worldwide. Honey bee visits, on average, constitute 13% of all floral visits in these networks, though their numerical importance varies considerably among the 56 networks in which they were recorded (range 0 – 85%). Honey bee importance is generally higher at sites with warmer and less variable temperatures and on mainland rather than island sites, but is unrelated to the honey bees' status as a native or introduced species. We find no evidence that floral visitation by honey bees in natural systems has declined in recent decades. In 46 networks that provide data on each studied plant species, honey bees account for the majority of floral visits to 15% of plant species, but fail to visit 63% of plant species. Using data from 35 plant species, we find that honey bees do not differ from the average non-honey bee floral visitor with respect to their single-visit ability to induce seed set, fruit set, or transfer pollen to stigmas. Therefore visitation frequency is a reasonable estimate of honey bee importance to pollination. In sum, while honey bees are the single most important pollinating species in natural habitats worldwide, these habitats remain highly dependent on non-honey bee pollinators for maintenance of pollination services.

INTRODUCTION

Animal-mediated pollination represents a vital terrestrial ecosystem function (Kearns and Inouye 1997, Ashman et al. 2004). Plants requiring animal pollination are diverse, comprising an estimated 87.5% of plant species worldwide (Ollerton et al. 2011). Animals engaged in pollination are also diverse (Fenster et al. 2004). While the diversity of pollinators is important for maintaining both the short-term functionality and the long-term stability of pollination services (Blüthgen and Klein 2011), at the local scale, the majority of pollination services may be performed by pollinator taxa that are numerically abundant and exhibit generalist foraging behavior (Vázquez et al. 2012, Winfree et al. 2014, Kleijn et al. 2015). Thus, understanding the population dynamics of taxa that occupy central roles in pollinator communities can provide insight into the fate of plant-pollinator interactions in a changing environment.

The western honey bee (Apidae: *Apis mellifera* L., hereafter "honey bee") is recognized as the most important pollinator species for many crops (Calderone 2012), though the role of non-honey bee pollinators in augmenting crop pollination has recently been emphasized (Garibaldi et al. 2011, 2013, Rader et al. 2016). Conversely, the importance of honey bees as pollinators in natural habitats remains a matter of debate (Butz Huryn 1997, Ollerton et al. 2012, Aebi et al. 2012). Given the large native range of the honey bee (spanning much of Europe, the Middle East, and Africa; Ruttner 1988), its long history of naturalization in its worldwide introduced range (Crane 1999), and rather early recognition of its potential to impact other pollinator taxa (Schaffer et al. 1983, Roubik 1983) and plants (Paton 1993, Butz Huryn 1997), it is surprising that only

recently have researchers begun to develop a broader understanding of the role of honey bees in non-agricultural ecosystems worldwide (Aslan et al. 2016, Geslin et al. 2017).

The prevalence of honey bees as potential pollinators in natural habitats is important for several reasons. First, factors that contribute to recent increases in the mortality of managed honey bee colonies (Goulson et al. 2015, Wilfert et al. 2016) may also affect feral or wild honey bees (Kraus and Page 1995, De la Rúa et al. 2009, Thompson et al. 2014). If honey bees are important pollinators of plant communities in natural systems, their decline could have implications for pollination services. Second, non-honey bee pollinators are declining in many parts of the world, largely driven by habitat loss and degradation, as well as other contributing factors such as pesticides, pathogens and parasites, invasion of non-native species, and climate change (Winfree et al. 2009, Potts et al. 2010, Goulson et al. 2015). In ecosystems impacted by anthropogenic disturbance, honey bees may help fill the pollination void left by declines in non-honey bee pollinators (Dick 2001, Xia et al. 2007, Junker et al. 2010, Hanna et al. 2013, Hermansen et al. 2014). Lastly, where honey bees reach high densities, as reported in some areas of their introduced range (Kato and Kawakita 2004, Abe et al. 2011, Giannini et al. 2015), they may exploit enough food resources (Cane and Tepedino 2016) to compete with other pollinators (Roubik et al. 1986, Thomson 2004, 2016, Goulson and Sparrow 2009). These phenomena are of broad ecological, evolutionary, and conservation interest, but to our knowledge, there currently exists no quantitative synthesis on the numerical importance of honey bees as floral visitors (i.e., as both pollinators and consumers of floral resources) in natural ecosystems worldwide, either in their native or introduced range.

Here, we use a meta-analysis to address the question of honey bee importance by taking advantage of a recent trend in pollination research—the documentation of community-level plant-pollinator interaction networks (hereafter "pollination networks"). Pollination network studies match the identity and frequency of each type of pollinator visiting each plant species within a locality (Memmott 1999). While these studies are performed to investigate a variety of questions (Albrecht et al. 2010, Rafferty and Ives 2011, Burkle and Knight 2012, Norfolk et al. 2015), data from pollination networks provide an excellent opportunity to investigate the importance of honey bees in natural habitats, not the least because the role of honey bees has rarely been their focus (Olesen et al. 2002, Kato and Kawakita 2004, Abe et al. 2011). Here, we compile a database of 80 pollination networks from natural and semi-natural habitats (i.e., excluding agricultural, urban, experimental or otherwise highly managed habitats) from all continents except Antarctica, as well as several oceanic islands, including regions where honey bees are native and places where they have been introduced. These networks allow us to address four questions regarding the ecological importance of honey bees in natural habitats. (1) What proportion of floral visits in natural habitats are due to honey bees? (2) What environmental factors determine the relative frequency of honey bees as floral visitors? (3) Do honey bees reach higher numerical dominance in their non-native range? (4) How are honey bee visits distributed among plant species? For instance, what proportion of plant species is not visited by honey bees, and for what proportion do honey bees contribute the majority of visits? Finally, network studies often use visitation frequency as a proxy for pollinator importance (Ballantyne et al. 2015). To further assess the value of honey bees as pollinators, we compile data on per-visit pollination efficiency (i.e., effect of single

visits by a pollinator species on seed set, fruit set, and/or pollen deposition) of honey bees relative to other floral visitors from studies on 35 plant species. Using these data, we address a fifth research question: (5) How does the per-visit pollination efficiency of honey bees compare to the average non-honey bee pollinator?

MATERIAL AND METHODS

Compilation of pollination networks: We used two approaches to compile our data set of pollination networks. First, we performed a literature search using the ISI Web of Science database with the search terms [pollinat* network], [pollinat* web], and [pollinat* visit* community] from October 2014 to August 2016. Second, we downloaded pollination network data from the Interaction Web DataBase of the National Center for Ecological Analysis and Synthesis website (https://data.nceas.ucsb.edu/) and the Web of Life Ecological Networks Database (http://www.web-of-life.es/). From the latter two databases, we downloaded all plant-pollinator interaction network datasets available as of December 2014 that reported visitation frequency in addition to the presence / absence of interaction between plant and pollinator taxa.

Each data point in our study consists of a weighted pollination network in which the set of interactions between each plant and pollinator pair is weighted by a measure of visitation frequency (i.e., number of total individuals observed contacting flowers or number of floral contacts per unit time). We defined a network as the sum of recorded plant-pollinator interactions in all study sites from a single study that fell within a 50-km diameter circle, regardless of the number of study plots that constitute the network. Sites within the same study that are separated by more than 50 km were treated as separate networks. When we encountered networks from different studies that were less than 50

km apart, we excluded those studies that sampled a smaller number of plants or pollinators, or documented fewer interactions.

All networks retained for analyses met the following criteria. The data were collected in natural or semi-natural habitats; agricultural, urban, experimental, or otherwise managed habitats were excluded. Each included network consisted of observations on five or more plant species when pooled across study sites; networks that focused only on select plant taxa with specialist pollination syndromes (e.g., oil-producing flowers) were excluded from analyses. Included networks documented a broad range of pollinators; studies that had a narrow taxonomic scope (e.g. social bees, bird pollinators with incidental observations of honey bees) or that explicitly excluded honey bees from data recording were excluded. Because we are primarily interested in quantifying the importance of honey bees in natural areas free of human interference, we excluded data from study sites (or entire studies) that are known to be heavily influenced by honey bee colonies stocked for adjacent agricultural pollination. Thus, our estimates of honey bee numerical importance may be conservative with respect to mosaic landscapes where natural habitats are intermixed with agriculture, but achieve a closer representation of the role of honey bees in natural areas worldwide, overall. We also did not exclude networks from localities outside of the honey bee's climatic niche, or where honey bees have never been introduced. In all, we obtained 80 networks (Table 4-S1) from 60 peer-reviewed studies, two graduate theses (Kevan 1970, Ingversen 2006), and our own study of plant-pollinator interactions in San Diego's scrub habitats (see Chapter 3 of this dissertation).

For each network, we obtained the following data from their associated publications or from study authors when data were not available from publications: latitude, longitude, and final year of data collection. When these data were not available and authors could not be reached, we used the approximate geographical center of the study locality listed in the publication, and the year of publication as the last year of data collection. We defined the native status of honey bees based on (Ruttner 1988); in Great Britain (United Kingdom), where the native status of honey bees is uncertain, we treated honey bees as native rather than introduced, but classifying honey bees there as introduced in that location did not substantially alter our results. We also extracted the following information from each study, when available: the proportion of total floral visits (or overall visitation frequency) contributed by honey bees, the proportion of plant species receiving at least one visit by honey bees, and the rank of honey bees with respect to both the total number of interactions (or overall interaction frequency) and the proportion of plant species visited. Additionally, we used geographic information system (GIS) analysis to obtain elevation data (http://www.gpsvisualizer.com/elevation) and bioclimatic variables ((Hijmans et al. 2005), http://www.worldclim.org) for each network based on its GPS coordinates. We also assigned each network as being on an island or a mainland; the latter category includes all continents as well as large islands > 200,000 km^2, namely Great Britain (United Kingdom), Honshu (Japan), and Greenland. For relevant studies for which raw data were not available, we contacted the corresponding authors to request data, or, in cases where data could not be shared, requested summary statistics on plant-pollinator interactions. When raw numeric data were unavailable from the publication or from authors, we used ImageJ to extract data from figures, where

possible (Table 4-S1). Due to the different methodologies and data-reporting requirements of each study, not all of the abovementioned variables were extracted from all networks.

Comparison of honey bees and bumble bees in pollination networks: Because studies vary in the level of detail with which individual species of floral visitors other than *Apis mellifera* are reported, we cannot reasonably compare frequencies of honey bee visitation with those of other single species across all of our networks. However, data are sufficiently detailed in 66 of our 80 networks to enable comparison of honey bees and bumble bees (Apidae: *Bombus*); the latter are the only other pollinator group with a similar pattern of local numerical abundance and widespread introduction compared to honey bees (Kearns and Inouye 1997, Russo 2016, Geslin et al. 2017). We compared the network-level relative visitation frequency of honey bees with that of all bumble bee species combined (as species-level identification was not provided in all datasets) using a paired *t*-test. Since our goal was to compare global patterns of numerical importance, this analysis did not exclude networks in which honey bees, bumble bees, or both taxa were absent. It is worth noting that the leafcutter bee *Megachile rotundata* (Fabricius), another widely introduced pollinator (Russo 2016, Geslin et al. 2017), was not reported in any of our 80 networks.

Drivers of honey bee visitation frequency among pollination networks worldwide: We used multiple linear regression models to examine environmental factors that may contribute to variation in the network-level frequency of floral visits by honey bees. Networks where honey bees were not recorded were excluded from this analysis because of the variety of reasons that could explain their absence, ranging from studies

that were outside the geographical or altitudinal range of the honey bee (e.g., Lundgren and Olesen 2005), to studies where honey bees were undetected despite being present in the ecosystem (Popic et al. 2013). Inclusion of networks that documented no honey bee visits using a zero-inflated multiple beta regression model (using package *gamlss* (Rigby and Stasinopoulos 2005) in Program R v.3.3.1 (R Development Core Team 2015)) did not qualitatively alter our results (Table 4-S2). The response variable in these regression models was the proportion of all floral visits (or overall visitation frequency) in each network contributed by honey bees, logit-transformed to improve normality (Warton and Hui 2011).

To identify the environmental model that best explains network-level honey bee visitation frequency, we generated models containing all possible combinations of the following explanatory variables (without interactions): latitude, longitude, altitude, land category (mainland *versus* island), and bioclimatic variables relating to temperature and precipitation (Hijmans et al. 2005). To incorporate bioclimatic variables, we first performed Principal Components Analysis (PCA) to avoid constructing models with highly collinear terms. We performed one PCA for the 11 variables measuring temperature (Table 4-S3), and a separate PCA for the eight bioclimatic variables measuring precipitation (Table 4-S4); these analyses enabled us to reduce bioclimatic variables to the first two principal components of the temperature variables (which together accounted for 87% of the variance; Table 4-S3) and the first two principal components of the precipitation variables (which together accounted for 89% of the variance; Table 4-S4). We used R package *glmulti* (Calcagno et al. 2010) to generate the candidate models and to select the best model using corrected Akaike's Information

Criterion (AICc) scores. We also used the resulting "best" environmental model to address the questions of whether or not the network-level frequency of honey bee visits depends on (1) their native status and (2) the year of data collection, by adding these two variables to the "best" environmental model, both individually and together.

Distribution of honey bee visitation frequency across plant species: We examined the distribution of honey bee relative visitation frequency across plant species as measured by the proportion of visits to each plant species contributed by honey bees. In this analysis, we included 46 networks in which (1) at least one visit by a honey bee was recorded, and (2) data on the proportion of total visits contributed by honey bees were available for each studied plant species. We pooled all plant species from all networks, and did not correct for cases in which the same plant species occurs in more than one network. Given the breadth of geographical areas and ecological contexts represented by networks in our study, the same plant species is expected to be served by different pollinator assemblages in distinct networks. Because plant species receiving few visits overall may tend to have extreme values of proportion of visits by honey bees, we also repeated this analysis after restricting the dataset to plant species with ≥ 10 visits recorded.

Pollination efficiency of honey bees: We used two approaches to compile our data set. First, we performed a literature search using the ISI Web of Science database with the search term [pollinat*] in combination with one of the following terms: [efficiency], [effectiveness], ["pollen deposition"], ["seed set"], ["fruit set"], or ["pollination biology of"], from October 2014 to August 2016. Second, we examined the literature cited sections of each of the studies found through the first approach for

additional studies that were not captured in the literature search. Data points in this analysis consist of studies of focal plant species that compared honey bees and at least one other pollinator taxon with respect to pollen deposition, seed set, or fruit set resulting from a single visit by an individual floral visitor (Ne'eman et al. 2009). In a small number of cases, we used ImageJ to extract data from figures when raw data were not available. In all, we obtained 33 studies reporting single-visit pollination efficiency data for 35 plant species, spanning 23 plant families (Table 4-S5). Of these, 19 plant species in 16 families were undomesticated, and 16 plant species in 7 families were grown in agricultural settings.

Multiple metrics of per-visit efficiency were available (e.g. number of pollen grains deposited, probability of fruit set, numbers of seed set) from some studies. We used or calculated seed set data whenever available since it is the most closely related to plant reproductive fitness (Cane and Schiffhauer 2003), fruit set when no seed counts were available, and pollen deposition when measures of seed and fruit set were unavailable. For each plant species in each study, we calculated the average single-visit pollination efficiency of non-honey bee pollinators as the numerical mean efficiency metric of all non-honey bee visitors studied. Then, we calculated the relative single-visit pollination efficiency of honey bees by dividing honey bee pollination efficiency by the average efficiency of non-honey bee floral visitors studied.

We used a one-sample *t*-test to test the null hypothesis that the pollination efficiency of honey bees equals the efficiency of the average non-honey bee floral visitor (i.e. whether the ratio of the efficiency of honey bees to that of the average non-honey bee floral visitor differs from one). Since honey bee relative efficiency did not differ

between agricultural and wild plant species (see Results and Discussion), data from all plant species were combined.

RESULTS AND DISCUSSION

Apis mellifera is the most important single species of pollinator in natural systems studied, owing to its wide distribution, considerable abundance, generalist foraging behavior, and competence as a pollinator. The honey bee was recorded in 16 of the 18 networks deemed to be within its native range and 38 of the 62 networks outside of its native range (Fig. 4-1, Table 4-S1). The mean network-level frequency of floral visits by honey bees was 12.64% (median = 1.56%) across all networks and 18.72% (median = 8.13%) among networks in which honey bees were recorded. Honey bees were the most frequent floral visitor in 17 networks and visited the most plant species in 14 networks (13 of which were networks in which honey bees also ranked first in visitation). In the 66 networks that provided data with sufficient resolution, the network-level visitation frequency of honey bees nearly exceeds that of all bumble bee species combined (*Apis* mean = 11.83%; *Bombus* mean = 6.42%; paired-*t* test $t_{65} = 1.84$, $P = 0.070$), further underscoring the numerical dominance of honey bees. Given that bumble bees are the only other pollinator guild comparable to honey bees with respect to both local importance and global distribution (Kearns and Inouye 1997, Russo 2016, Geslin et al. 2017), it seems unlikely that any other single pollinator species contends with honey bees with respect to worldwide numerical importance. These findings are consistent with the current view that honey bees are super-generalists that occupy central roles in pollination networks (Giannini et al. 2015, Aslan et al. 2016, Geslin et al. 2017).

The numerical importance of honey bees is predicted by environmental context. The best multiple regression model selected from a set of candidate models of environmental variables revealed that the network-level frequency of visits by honey bees is positively related to the first principal component of temperature bioclimatic variables (Table 4-S3), where higher values correspond with higher overall temperature, higher isothermality, lower annual range and lower seasonality (Table 4-1). Honey bees were also more frequent floral visitors in mainland networks compared to island networks (Table 4-1). Perhaps surprisingly, our regression model revealed no effect of the honey bee's native status on honey bee numerical importance (Table 4-1). Release from pathogens and parasites often contributes to the success of introduced species (Torchin et al. 2003); this factor may be unimportant in honey bees because many of their pathogens have spread worldwide due to trafficking of domestic colonies (Goulson et al. 2015, Wilfert et al. 2016). Nevertheless, it is noteworthy that eight of the ten networks with the highest relative frequency of honey bee visits come from introduced range localities, and that in five of these networks, honey bees accounted for more than half of the total visits recorded (Olesen et al. 2002, Aguiar 2003, Kato and Kawakita 2004, Abe et al. 2011, Hung et al. 2017). While Abe et al. (Abe et al. 2011) found that honey bee dominance in the Ogasarwara satellite islands was driven by an introduced lizard's preferential predation on native pollinators, further studies are needed to understand why honey bees reach high abundance in some parts of their introduced range, but not others.

Also surprising is our finding that study year was unrelated to honey bee numerical importance (Table 4-1), given the high mortality in managed honey bee colonies reported over the last two decades (vanEngelsdorp et al. 2008, 2009). Agents

responsible for increased mortality in managed colonies can also affect wild or feral honey bee colonies (Kraus and Page 1995, De la Rúa et al. 2009, Thompson et al. 2014), but ongoing research also reveals the resilience of unmanaged honey bee populations to mortality agents such as parasites and pathogens (Mikheyev et al. 2015, Loftus et al. 2016). In our pollination networks, the degree to which honey bee individuals are coming from managed *versus* unmanaged colonies likely varies based on geographical location and proximity of the study site to agriculture. However, in one network with high honey bee numerical importance (Hung et al. 2017), genetic testing indicated that the majority of the honey bee foragers were derived from feral, Africanized hives (Kono and Kohn 2015).

Although honey bees are numerically dominant pollinators in many networks, their importance as floral visitors to individual plant species varies widely. An examination of 46 pollination networks that provide data on each studied plant species yielded 1629 plant taxa within these networks. While some plant taxa species are found in more than one network, we treat each plant species within each network independently because our goal is to examine the frequency with which honey bees visit each plant species within discrete communities. Across these 1629 plant taxa, we found a strongly, positively skewed distribution of honey bee visitation frequency (Fig. 4-2A). Honey bees were the only documented visitors of 5.34% of plant taxa (mean among networks = 5.03%, median = 0%), and contributed the majority (≥ 50%) of visits to 15.16% of plant taxa (mean among networks = 14.29%, median = 4.27%). However, honey bees also failed to visit the majority (63.35%) of plant taxa (mean among networks = 59.54%, median = 60.65%). Restricting the analysis to plant taxa with ≥ 10 visits recorded to

minimize extreme values due to low sample size did not qualitatively affect our results (Fig. 4-2B). In this data subset, honey bees were the sole documented visitors of 3.44% of plant taxa (mean among networks = 4.53%, median = 0%), contributed the majority (≥ 50%) of visits to 17.84% of plant taxa (mean among networks = 17.73%, median = 0%), and failed to visit 50.31% of plant taxa (mean among networks = 49.15%, median = 46.69%).

Our finding that honey bees numerically dominated a number of plant taxa is perhaps unsurprising given their ability to recruit nest mates to spatially and temporally abundant floral resources (von Frisch 1967). However, it is noteworthy that this pattern holds true in their introduced range, where floral resources monopolized by honey bees presumably coevolved with native pollinators. This analysis cannot distinguish whether honey bees dominate certain floral resources because they displace other pollinators (via exploitative or interference competition) or because they have the ability to profit from floral resources not valued by other pollinators. However, the data do suggest that honey bees possess the potential to disrupt interactions between plants and other pollinators in the majority of natural communities in which they occur. On the other hand, our finding that honey bees are frequent floral visitors to only a small subset of the plant species in a community is consistent with studies investigating honey bee colony-level resource use (de Vere et al. 2017) and underscores the importance of maintaining robust, diverse communities of non-honey bee pollinators for the persistence of the majority of plant species in natural communities.

While our analyses of pollination networks worldwide reveal that honey bees are exceptionally abundant and generalized floral visitors, our analysis of pollination

efficiency of honey bees reveals that they are average pollinators with respect to their pollination efficiency (Fig. 4-3). Using a dataset of 35 plant species spanning 23 plant families (Table 4-S5) that exhibit a diversity of flower sizes, shapes, and colors, we compared honey bees and non-honey bee floral visitors with respect to seed set, fruit set, or pollen deposition resulting from single floral visits (Ne'eman et al. 2009). The relative pollination efficiency of honey bees did not differ between the 16 agricultural and 19 non-agricultural plant species (Welch's two-sample *t*-test, $t_{30.75} = 0.80$, $P = 0.43$), perhaps because flowers of agricultural species (e.g. almonds (*Prunus dulcis*), squash (*Cucurbita pepo*), tomato (*Solanum lycopersicum*)) often closely resemble those of their wild relatives. Overall, we found no evidence that the pollination efficiency of honey bees consistently differs from that of the average of the non-honey bee floral visitors considered in these studies (one sample *t*-test, $t_{34} = 0.39$, $P = 0.70$; mean relative efficiency of honey bee = 96.5% that of the average non-honey bee pollinator).

Since the importance of a particular pollinator to a given plant species is often calculated as its per-visit efficiency multiplied by its visitation frequency (Vázquez et al. 2012), it seems reasonable, given our results, to assume that the ecological importance of honey bees as pollinators in any community is satisfactorily estimated by their visitation frequency. However, since honey bees are known to exhibit poor efficiency at pollinating certain plant taxa (Westerkamp 1991, Aslan et al. 2016), we caution that careful studies are needed to demonstrate the importance of honey bees as pollinators to particular plant species. Further, in at least one case, high visitation frequency by a pollinator (the introduced bumblebee *Bombus terrestris* (L.)) damaged raspberry flowers and led to reduced reproductive success (Sáez et al. 2014). On plant species and in plant

communities where honey bees reach high visitation rates, a similar negative relationship between visitation frequency and plant reproductive fitness may occur and is worthy of investigation (Aizen et al. 2014).

As a numerically dominant, super-generalist pollinator, honey bees may influence the fitness (Thomson 2016) and behavior (Hansen et al. 2002) of competing pollinators, enhance (Dick 2001) as well as reduce (Gross and Mackay 1998) plant fitness, and facilitate the spread of non-native weeds (Barthell et al. 2001) and pathogens (Graystock et al. 2016). Given the ecological importance of honey bees, there is little doubt that changes in their distribution (e.g., via introductions or local extirpations) and abundance will impact the evolutionary trajectory of co-occurring mutualists (Mu et al. 2014) and competitors, and likely the long-term eco-evolutionary dynamics of communities in which they take part. Our results underscore the urgent need for more data on how honey bees, and the potential loss thereof, shape the ecology and evolution of plant-pollinator interactions on global and local scales.

ACKNOWLEDGMENTS

We gratefully acknowledge the following individuals who provided raw data, key summaries of data, and/or helpful discussions on the use of their data: T. Abe, R. Alarcón, J. Albrecht, I. Bartomeus, J. Bascompte, N. Blüthgen, L. Burkle, M. Campos-Navarrete, L. Carvalheiro, A. Gotlieb, M. Hagen, S. Hegland, M. Koski, X. Loy, H. Marrero, A. Nielsen, O. Norfolk, N. Rafferty, D. Robson, R. Ramos-Jiliberto, K. Trøjelsgaard, C. Tur, D. Vázquez, and Y. Yoshihara. We also thank all authors who have made their raw data publicly available. We are also grateful to L. Levin, J. Minster, and J. Nieh for providing

constructive feedback on our manuscript. This work was funded by the NSF Doctoral Dissertation Improvement Grant DEB-1501566; the Mildred E. Mathias Graduate Student Research Grant and the Institute for the Study of Ecological & Evolutionary Climate Impacts Graduate Fellowship from the University of California Natural Reserve System; the Frontiers of Innovation Scholar Fellowship, the Academic Senate Grant, and the McElroy Fellowship from the University of California, San Diego; the Sea and Sage Audubon Society Bloom-Hays Ecological Research Grant; and the California Native Plants Society Educational Grant.

Chapter 4, in part, is currently being prepared for submission for publication of the material. Hung, Keng-Lou J.; Kingston, Jennifer M.; Albrecht, Matthias; Holway, David A.; Kohn, Joshua R. The dissertation author was the primary investigator and author of this manuscript.

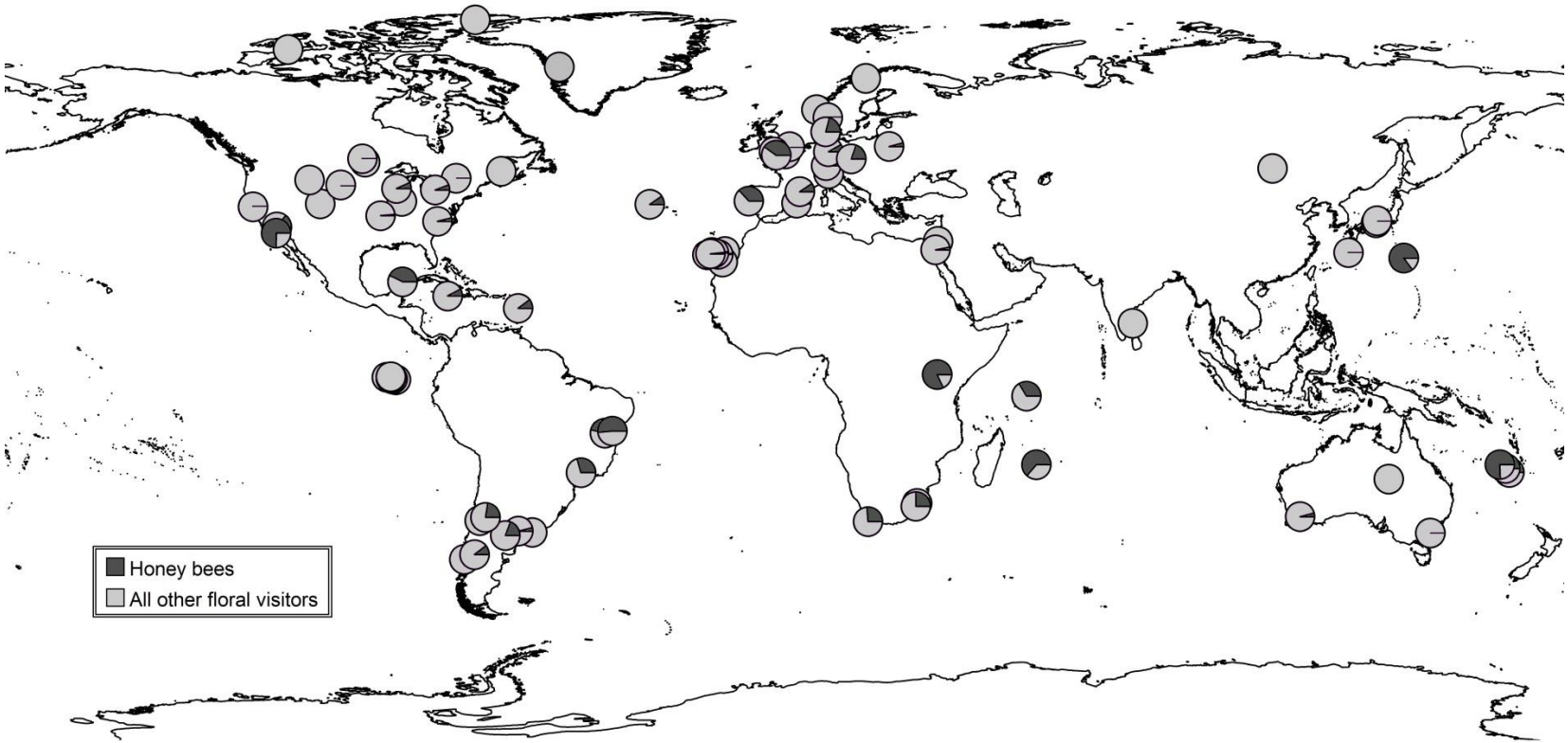

Figure 4-1. Relative visitation frequencies of the western honey bee in 80 plant-pollinator interaction networks in natural habitats worldwide. The western honey bee is generally considered a native species in Europe, the Middle East, and Africa; it is considered to be introduced in all other localities.

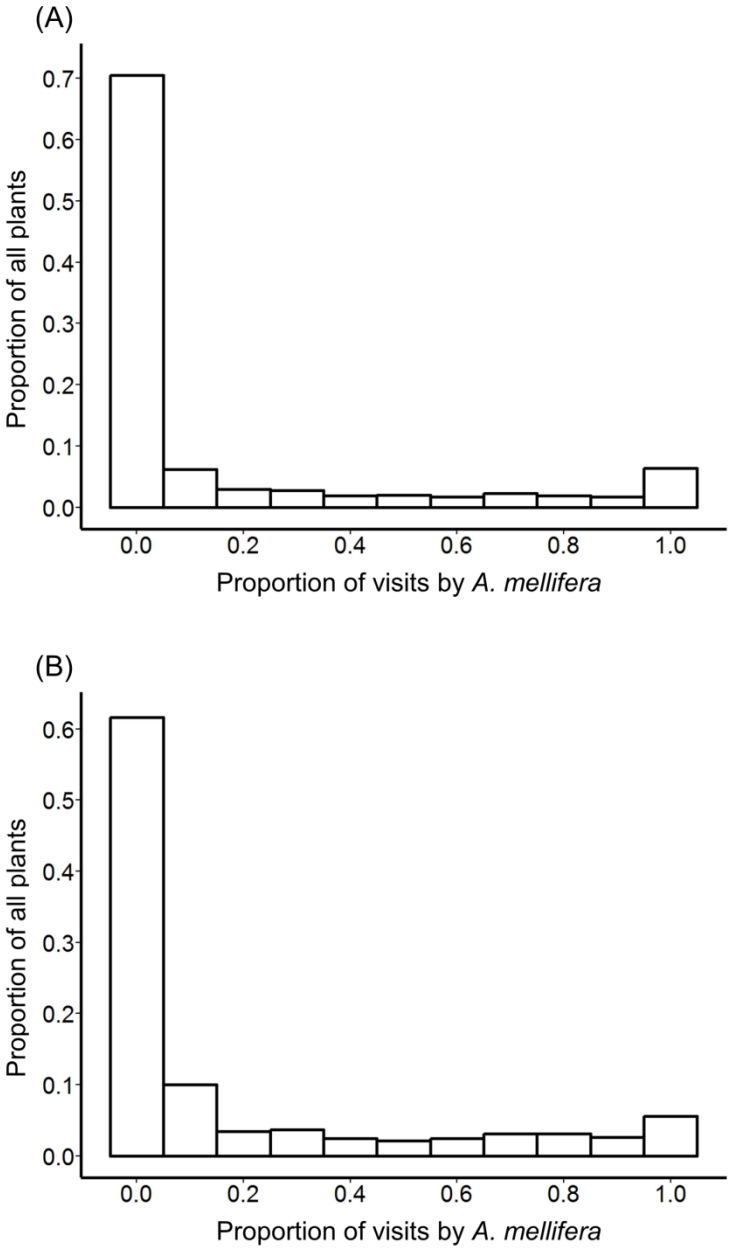

Figure 4-2. The distribution of plant species with respect to the proportion of their total recorded floral visits contributed by honey bees. Each data point represents one plant species in a distinct network; thus plant species present in multiple networks are included multiple times as separate data points. Plots show (A) 1,629 plant taxa from 42 plant-pollinator interaction networks where honey bees were documented, and (B) 813 plant taxa with ≥ 10 recorded pollinator visits, from 40 plant-pollinator interaction networks where honey bees were documented.

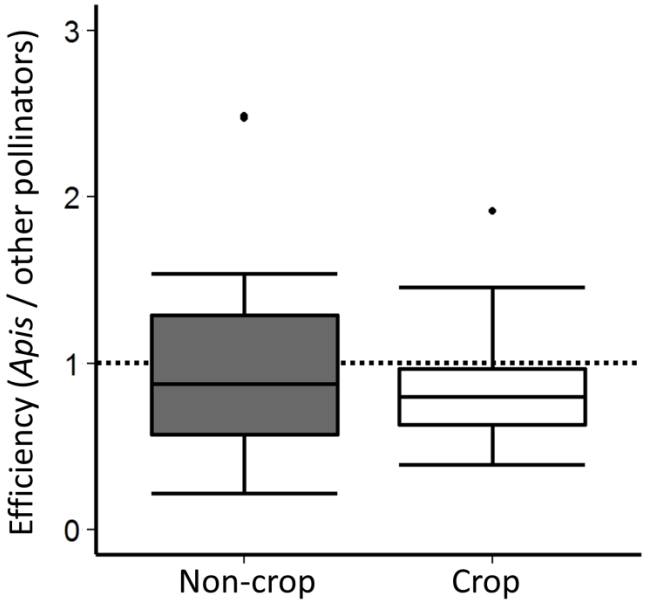

Figure 4-3. Relative efficiency of honey bees compared to other studied pollinators with respect to the ability to deposit pollen or effect fruit or seed set with a single visit. For each plant species studied, honey bee relative efficiency is calculated by dividing the per-visit efficiency metric of honey bees by the mean of efficiency metrics of all non-honey bee taxa.

Table 4-1. The best multiple linear regression model relating environmental variables to honey bee relative visitation frequency in plant-pollinator interaction networks worldwide (n = 54 networks where honey bees were present), selected based on corrected AIC scores from candidate models that included all possible combinations of the following independent variables: latitude, longitude, altitude, land category (mainland *versus* island), the first two principle components of 11 temperature-related bioclimatic variables, and the first two principle components of eight precipitation-related bioclimatic variables. Temperature PC1 is positively correlated with overall temperature and isothermality, and negatively correlated with temperature seasonality and annual temperature range. Models examining the influence of honey bee native status and last year of study on honey bee relative visitation frequency were constructed by adding these two variables one at a time, as well as both together, to the best linear model of environmental variables.

Model (Δ AICc) / Variable	**Estimate**	**Test stat.**	***P* value**
Best environmental model ("BEM") (Δ AICc = 0)	Adj. $R^2 = 0.28$	$F_{2,51} = 11.17$	< 0.001
Temperature PC1	$\beta = 0.70$	$t = 4.68$	< 0.001
Land category (mainland = 1, island = 0)	$\beta = 1.40$	$t = 2.12$	0.04
BEM + *Apis* native status (Δ AICc = 1.11)	Adj. $R^2 = 0.28$	$F_{3,50} = 7.90$	< 0.001
Temperature PC1	$\beta = 0.71$	$t = 4.77$	< 0.001
Land category (mainland = 1, island = 0)	$\beta = 1.22$	$t = 1.79$	0.08
Apis native status (native = 1, introduced = 0)	$\beta = 0.65$	$t = 1.11$	0.27
BEM + last study year (Δ AICc = 2.02)	Adj. $R^2 = 0.27$	$F_{3,50} = 7.49$	0.003
Temperature PC1	$\beta = 0.68$	$t = 4.44$	< 0.001
Land category (mainland = 1, island = 0)	$\beta = 1.37$	$t = 2.04$	0.05
Last study year (years CE)	$\beta = 0.02$	$t = 0.62$	0.54
BEM + *Apis* nat. stat. + last study yr. (Δ AICc = 3.42)	Adj. $R^2 = 0.27$	$F_{4,49} = 5.88$	< 0.001
Temperature PC1	$\beta = 0.70$	$t = 4.53$	< 0.001
Land category (mainland = 1, island = 0)	$\beta = 1.20$	$t = 1.75$	0.09
Apis native status (native = 1, introduced = 0)	$\beta = 0.60$	$t = 1.02$	0.31
Last study year (years CE)	$\beta = 0.02$	$t = 0.45$	0.65

APPENDIX 4-1. Pollination networks included in the meta-analysis.

Table 4-S1. Pollination networks (n = 80) used in addressing research questions 1 through 4. Land category classifications are mainland ("M") and island ("I"). "Proportion plants *Apis*" refers to the proportion of plant species studied in the network that received at least one visit from *Apis mellifera*. "Proportion visits *Apis*" refers to the proportion of all floral visits documented in the network contributed by *A. mellifera*. Distinct localities reported in the same study (i.e., localities separated by > 50 km) are listed separately with the locality denoted in parentheses. Full citations are listed below in the same order as the studies.

	Study	Lat.	Lon.	Land cat.	*Apis* native?	Proportion plants *Apis*	Proportion visits *Apis*
1.	Abe 2011	27.07	142.21	I	No	0.5774	0.8511
2.	Aguiar 2003	-12.7	-39.77	M	No	0.64	0.5135
3.	Alarcon 2008	34.22	-116.95	M	No	0.4107	0.1441
4.	Albrecht J. 2014	52.70	23.85	M	Yes	0.8235	0.0403
5.	Albrecht M. 2010	46.44	9.94	M	No	0	0
6.	Andena 2005	-22.25	-47	M	No	0.4242	0.2957
7.	Arroyo 1982	-33.28	-70.27	M	No	0	0
8.	Barret 1987	47.02	-65.35	M	No	0	0
9.	Bartomeus 2008	42.32	3.32	M	Yes	0.4063	0.0929
10.	Bartomeus 2010	51.5	9.9	M	Yes	0.3871	0.0837
11.	Burkle 2009	39.51	-106.97	M	No	0	0
12.	Burkle 2012	36.8	-93.1	M	No	0.2222	0.0153
13.	Campos-Navarrete 2013	21.61	-88.04	M	No	0.65	0.4264
14.	Carvalheiro 2008	51.46	-2.64	M[4]	Yes[5]	0.0313	0.0064
15.	Chacoff 2012[1]	-32.54	-68.96	M	No	0.4746	0.224
16.	Dicks 2002	52.67	1	M[4]	Yes[5]	0.0435	0.0034
17.	Dupont 2009	56.07	9.28	M	Yes	N/A	0.2088
18.	Elberling 1999	68.35	18.5	M	No	0	0
19.	Ferrero 2013	40.165	-8.41	M	Yes	0.8571	0.365[8]
20.	Forup 2008	50.71	-2.11	M[4]	Yes[5]	0.7143	0.4017
21.	Gibson 2012	-33.5	19	M	Yes	0.75	0.26[9]
22.	Gotlieb 2011	30.9	35.1	M	Yes	0	0
23.	Grass 2013	-30	30	M	Yes	0.5273	0.2497
24.	Hagen 2010	0.28	34.9	M	Yes	0.6471	0.819
25.	Hegland 2010	61.16	7.17	M	No	0	0
26.	Hung 2015[2]	32.8	-117.1	M	No	0.8039	0.7610
27.	Ingverson 2006 (Dominica)[2]	15.52	-61.46	I	No	0.3548	0.101
27.	Ingverson 2006 (Jamaica)[2]	18.35	-77.65	I	No	0.2069	0.0788
28.	Inoue 1990	35.17	135.87	M[4]	No	0	0
29.	Inouye 1988	-36.15	148.33	M	No	0.025	0.0023

Study	Lat.	Lon.	Land cat.	*Apis* native?	Proportion plants *Apis*	Proportion visits *Apis*
30. Janovsky 2013	49.85	15.15	M	Yes	0.375	0.1946
31. Johnson 2009	-29.32	30.28	M	Yes	0.4	0.0591
32. Kaiser-Bunbury 2011	-4.67	55.43	I	No	0.8462	0.335
33. Kato 1996	35.65	136.08	M[4]	No	0.0469	0.0043
34. Kato 2000	28.3	129.5	I	No	0.0181	0.0009
35. Kato 2004 (Central NC)	-21.3	165.6	I	No	0.5385	0.4741
35. Kato 2004 (Northern NC)	-20.4	164.3	I	No	0.7222	0.7561
35. Kato 2004 (Southern NC)	-22.2	166.5	I	No	0.3721	0.2967
36. Kevan 1970[2]	81.82	-71.3	I	No	0	0
37. Koski 2015	38.86	-122.41	M	No	0.1176	0.0086
38. Larson 2016	43.88	-102.28	M	No	0.0962	0.0068
39. Loy 2015	-32.39	118.38	M	No	0.4286	0.0422
40. Lundgren 2005	71	-52	M[4]	No	0	0
41. Marrero 2014 (Carlos)	-35.7	-61.4	M	No	0.23	0.0393
41. Marrero 2014 (Pila)	-36	-58.2	M	No	0	0
41. Marrero 2014 (Toay)	-36.7	-64.4	M	No	0.2663	0.1923
42. Memmot 1999	51	0	M[4]	Yes[5]	0.04	0.0023
43. Morales 2006	-41.1	-71.5	M	No	0.4815	0.1044
44. Moreira 2015	-13.18	-41.5	M	No	N/A	0.464
45. Mosquin 1967	75	-114.38	I	No	0	0
46. Motten 1986	35.5	-80	M	No	0.4615	0.0391
47. Nayak 2010	12.07	79.88	M	No	0	0
48. Neilsen 2014	59.35	9.75	M	No[6]	0.1	0.0058
49. Norfolk 2015	28.95	34.54	M	No[7]	0.2	0.0286
50. Olesen 2002 (Aigrettes)	-20.42	57.73	I	No	0.857	0.6369
50. Olesen 2002 (Flores)	39.4	-31.2	I	No	0.3	0.0939
51. Popic 2013	-23.75	138.75	M	No	0	0
52. Rafferty 2011	43.04	-89.43	M	No	0.5	0.0316
53. Robson 2008 (LPM)	49.89	-97.27	M	No	0.0714	0.0075
53. Robson 2008 (TGPP)	49.12	-96.66	M	No	0	0
54. Schemske 1978	40.11	-88.2	M	No	0	0
55. Simanonok 2014	45	-109.42	M	No	0	0
56. Small 1976	45.42	-75.7	M	No	0.3846	0.0247
57. Smith-Ramirez 2005	-42.19	-73.81	I	No	0.6923	0.096
58. Taki 2007	42.72	-80.53	M	No	0.21429	0.057
59. Traveset 2013 (S. Cristobal)	-0.89	-89.6	I	No	0	0
59. Traveset 2013 (S. Cruz)	-0.75	-90.32	I	No	0	0
59. Traveset 2013 (Fernandina)	-0.33	-91.63	I	No	0	0
59. Traveset 2013 (Pinta)	-0.56	-90.73	I	No	0	0
59. Traveset 2013 (Santiago)	-0.22	-90.73	I	No	0	0
60. Trøjelsgaard 2015 (Fuerte V.)[3]	28.56	-13.89	I	No	0	0

Study	Lat.	Lon.	Land cat.	*Apis* native?	Proportion plants *Apis*	Proportion visits *Apis*
60. Trøjelsgaard 2015 (Gomero)[3]	28.04	-17.23	I	No	0.2667	0.0158
60. Trøjelsgaard 2015 (G. Canaria)[3]	27.9	-15.43	I	No	0.4167	0.011
60. Trøjelsgaard 2015 (Hierro)[3]	27.8	-17.9	I	No	0	0
60. Trøjelsgaard 2015 (Tenrife S.)[3]	28.22	-16.42	I	No	0.375	0.0143
60. Trøjelsgaard 2015 (Tenrife T.)[3]	28.35	-16.91	I	No	0.1053	0.002
60. Trøjelsgaard 2015 (W. Sahara)[3]	26.16	-14.42	M	Yes	0	0
61. Tur 2013	39.7	2.6	I	Yes	0.25	0.057
62. Weiner 2011	48.3	9.4	M	Yes	0.349	0.0655
63. Yoshihara 2008	47.66	112.04	M	No	0	0

[1] Diego P. Vázquez provided two additional years of data collected using the same protocol as that reported in this study.
[2] Data were from thesis chapters not published in peer-reviewed journals.
[3] Data from this study were obtained from the Dryand Digital Repository: Trøjelsgaard, K., P. Jordano, D.W. Carstensen, and J.M. Olesen. 2015. Data from: Geographical variation in mutualistic networks: similarity, turnover and partner fidelity. url: http://dx.doi.org/10.5061/dryad.76173
[4] We define islands as having surface area < 200,000 km^2; therefore Great Britain (United Kingdom), Honshu (Japan), and Greenland are considered mainland sites.
[5] The native status of western honey bees in Great Britain is debated; we chose to analyze them as a native species.
[6] Honey bees in this locality appear to have derived from colonies originally introduced for honey production; thus we chose to analyze them as an introduced species.
[7] Honey bees are native to the greater regional biome but have only recently been introduced to this locality; thus we chose to analyze them as an introduced species.
[8] Honey bee relative visitation frequency was extracted from Fig. 2 of publication using ImageJ. Only data from sites without *Oxalis pes-caprae* weeding treatment were included in the analysis.
[9] Honey bee relative visitation frequency was extracted from Fig. 2 of publication using ImageJ. Only data from sites not invaded by *Acacia saligna* were included in the analysis.

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APPENDIX 4-2. Supplemental analyses.

Table 4-S2. The best zero-inflated multiple beta regression model relating environmental variables to the distribution of honey bee relative visitation frequency in plant-pollinator interaction networks worldwide (n = 79 networks where bioclimatic variables were available), selected based on corrected AIC scores from the same candidate models as those used in our main analysis (see Methods and Table 4-1). Models examining the influence of honey bee native status and last year of study on honey bee relative visitation frequency were constructed by adding these two variables one at a time, as well as both together, to the best linear model of environmental variables.

Model (Δ AICc) / Variable	**Estimate**	***t* value**	***P* value**
Best environmental model ("BEM") (Δ AICc = 0)			
Temperature PC1	0.39367	4.237	< 0.001
Land category (mainland = 1, island = 0)	0.81121	2.265	0.026
μ coefficient intercept (link = logit)	-2.25566	6.528	< 0.001
σ coefficient intercept (link = log)	1.2246	6.042	< 0.001
nu coefficient intercept (link = logit)	-0.7701	3.184	0.002
BEM + *Apis* native status (Δ AICc = 1.39)			
Temperature PC1	0.40936	4.309	< 0.001
Land category (mainland = 1, island = 0)	0.74207	2.039	0.045
Apis native status (native = 1, introduced = 0)	0.31128	0.985	0.33
μ coefficient intercept (link = logit)	-2.31568	6.607	< 0.001
σ coefficient intercept (link = log)	1.2505	6.119	< 0.001
ν coefficient intercept (link = logit)	-0.7701	3.184	0.00214
BEM + last study year (Δ AICc = 2.25)			
Temperature PC1	0.38795	4.751	< 0.001
Land category (mainland = 1, island = 0)	0.80985	2.264	0.026
Last study year (years CE)	0.00555	0.308	0.76
μ coefficient intercept (link = logit)	-13.37819	0.371	0.71
σ coefficient intercept (link = log)	1.2249	7.669	< 0.001
ν coefficient intercept (link = logit)	0.7701	3.184	0.002
BEM + Apis nat. stat. + last study yr. (Δ AICc = 3.75)			
Temperature PC1	0.405015	4.954	< 0.001
Land category (mainland = 1, island = 0)	0.741535	2.029	0.046
Apis native status (native = 1, introduced = 0)	0.304142	0.955	0.34
Last study year (years CE)	0.004096	0.225	0.82
μ coefficient intercept (link = logit)	-10.523151	0.289	0.77
σ coefficient intercept (link = log)	1.25	7.817	< 0.001
ν coefficient intercept (link = logit)	-0.7701	3.184	0.002

Table 4-S3. Results of principal components analysis of 11 bioclimatic variables describing patterns in temperature. The first two principle component axes were used as independent variables in constructing the environmental model explaining patterns of honey bee numerical importance in networks worldwide.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	2.714	1.456	1.014	0.624	0.248	0.152
% of variance explained	66.97%	19.28%	9.35%	3.54%	0.06%	0.02%
Cumulative variance explained	66.97%	86.25%	95.60%	99.14%	99.70%	99.91%
Axis loadings						
Mean annual temp.	0.362	-0.108	-0.077	0.073	-0.039	-0.219
Mean temp. warmest quarter	0.302	-0.337	-0.255	0.183	-0.263	-0.115
Mean temp. coldest quarter	0.367	0.044	0.023	0.037	0.103	-0.215
Mean temp. wettest quarter	0.248	-0.312	-0.393	-0.668	0.339	0.350
Mean temp. driest quarter	0.342	0.083	0.148	0.469	0.107	0.784
Mean diurnal temp. range	-0.056	-0.496	0.655	-0.010	0.462	-0.081
Max. temp. of warmest month	0.256	-0.476	-0.066	0.262	-0.115	-0.175
Min. temp. of coldest month	0.362	0.117	-0.047	0.065	0.072	-0.155
Temp. annual range	-0.286	-0.429	0.017	0.079	-0.157	0.083
Temp. isothermality	0.290	0.014	0.516	-0.455	-0.648	0.137
Temp. seasonality	-0.315	-0.313	-0.221	0.086	-0.341	0.260

Table 4-S4. Results of principal components analysis of eight bioclimatic variables describing patterns of precipitation. The first two principle component axes were used as independent variables in constructing the environmental model explaining patterns of honey bee numerical importance in networks worldwide.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	2.419	1.118	0.817	0.408	0.221	0.093
% of variance explained	73.14%	15.63%	8.34%	2.08%	0.06%	0.01%
Cumulative variance explained	73.14%	88.77%	97.11%	99.19%	99.80%	99.91%
Axis loadings						
Annual precip.	-0.409	0.104	-0.027	0.060	-0.125	-0.788
Precip. of wettest month	-0.372	0.358	-0.083	0.295	-0.440	0.335
Precip. of driest month	-0.381	-0.286	0.035	-0.493	-0.221	0.425
Precip. of wettest quarter	-0.377	0.337	-0.093	0.314	-0.049	0.138
Precip. of driest quarter	-0.388	-0.261	0.067	-0.415	-0.197	-0.219
Precip. of warmest quarter	-0.351	-0.022	-0.619	-0.069	0.666	0.089
Precip. of coldest quarter	-0.305	0.211	0.763	-0.017	0.506	0.090
Precip. seasonality	0.201	0.744	-0.112	-0.625	-0.027	-0.050

APPENDIX 4-3. Plant species included in meta-analysis of single-visit pollination efficiency.

Table 4-S5. Plant species (n = 35) used in assessing the single-visit pollination efficiency of honey bees relative to the average floral visitor for a focal plant species. *Apis* relative efficiency is calculated by dividing the single-visit pollination efficiency measure of honey bees by the mean efficiency measure of all other pollinators studied on the plant species in question. Full citations are listed below in the same order as the studies.

Study	Plant species	Plant family	Crop	Metric	*Apis* rel. eff.
1. Albano 2009	*Fragaria ananassa*	Rosaceae	Yes	Seed set	0.9648
2. Bruckman 2014	*Phacelia parryi*	Boraginaceae	No	Seed set	0.5693
3. Cane 2003	*Vaccinium macrocarpon*	Ericaceae	Yes	Seed set[3]	0.4331
4. Canto-Aguillar 2000	*Cucurbita moshata*	Curcubitaceae	Yes	Pollen deposition	0.7697
5. Dieringer 1992	*Agalinis strictifolia*	Scrophulariaceae	No	Seed set	1.4058
6. Fagua 2011	*Melocactus intortus*	Cactaceae	No	Fruit set	0.9901
7. Faria 2015	*Psychotria carthagenensis*	Rubiaceae	No	Fruit set	0.8755
8. Freitas 1998	*Anacardium occidentale*	Anacardiaceae	No[1]	Seed set	0.8776
9. Fumero-Caban 2007	*Pitcairnia angustifolia*	Bromeliaceae	No	Pollen deposition	0.8605
10. Gross 1998	*Melastoma affine*	Melastomataceae	No	Fruit set	0.2174
11. Gross 2001	*Dillwynia juniperina*	Fabaceae	No	Fruit set	0.5800
12. Javorek 2002	*Vaccinium angustifolium*	Ericaceae	Yes	Pollen deposition	0.3915
13. Junker 2010	*Metrosideros polymorpha*	Mytraceae	No	Pollen deposition	2.4713
14. Keys 1995	*Prosopsis veluntia*	Fabaceae	No	Fruit set	0.8627
15. Macias-Macias 2009	*Solanum lycopersicon*	Solanaceae	Yes	Seed set[4]	0.5852
15. Macias-Macias 2009	*Capsicum chinense*	Solanaceae	Yes	Seed set[4]	0.6143
16. Monzon 2004	*Pyrus communis*	Rosaceae	Yes	Fruit set	0.7326
17. Osorio-Beristain 1997	*Kallstroemia grandiflora*	Zygophyllaceae	No	Pollen deposition	0.4063
18. Pan 2013	*Hedysarum scoparium*	Fabaceae	No	Pollen deposition	1.1207
19. Park 2016	*Malus domestica*	Rosaceae	Yes	Fruit set	0.7475
20. Rader 2009	*Brassica rapa*	Brassicacae	Yes	Pollen deposition	1.4574
21. Rader 2013	*Citrullus lanatus*	Curcubitaceae	Yes	Pollen deposition	0.9383
22. Romero 2013	*Jatropha curca*	Euphorbaceae	Yes[2]	Fruit set	0.9744

	Study	Plant species	Plant family	Crop	Metric	*Apis* rel. eff.
23.	Stoepler 2012	Asclepias exaltata	Apocynaceae	No	Pollen deposition	1.5391
24.	Sun 2013	*Pedicularis densispica*	Orobanchaceae	No	Pollen deposition	0.4000
25.	Tepedino 1981	*Cucurbita pepo*	Curcubitaceae	Yes	Fruit set	0.9198
26.	Thomson 2001	*Prunus dulcis*	Rosaceae	Yes	Pollen deposition	1.3295
27.	Watts 2012	*Duranta mandonii*	Verbanaceae	No	Fruit set	2.4842
28.	Welsford 2012	*Wahlenbergia cuspidata*	Capanulaceae	No	Seed set[4]	1.4138
28.	Welsford 2012	*Wahlenbergia krebsii*	Capanulaceae	No	Seed set[4]	0.5110
29.	Willmer 2014	*Geranium sanguineum*	Geraniaceae	No	Pollen deposition	0.7926
30.	Willmer 1994	*Rubus idaeus*	Rosaceae	Yes	Pollen deposition	0.6395
31.	Wist 2013	*Echinacea angustifolia*	Asteraceae	Yes[2]	Pollen deposition[5]	1.9124
32.	Young 2007	*Impatiens capensis*	Balsaminaceae	No	Seed set	1.1695
33.	Zhang 2015	*Prunus persica*	Rosaceae	Yes	Pollen deposition	0.8253

[1] *Anacardium occidentale* (cashew) was analyzed as a non-agricultural species because studied plants were in a wild population occurring in the species' native range.
[2] *Jatropha curca* (physic nut) and *Echinacea angustifolia* were analyzed as agricultural species because studied plants occurred in managed monocultures.
[3] Seed set was estimated as the predicted seeds per 100 visits for each pollinator species based on empirically measured pollen saturation curve.
[4] Seed set was calculated as the product of seeds per fruit and percent fruit set measured for each pollinator species.
[5] Pollen deposition was estimated as the proportion of disc flowers in a capitulum with retracted style and pollen tubes at style base after a single visit by a pollinator species.

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