HOW DO SPECIES ABUNDANCE DISTRIBUTIONS INFLUENCE PLANT – POLLINATOR NETWORKS?

By

MOLLY KATHERINE MacLEOD

A dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Ecology and Evolution

Written under the direction of

Rachael Winfree

And approved by

New Brunswick, New Jersey

January 2016

ABSTRACT OF THE DISSERTATION

How do species abundance distributions influence plant – pollinator networks? By MOLLY MacLEOD

Dissertation director: Rachael Winfree

Plant – pollinator networks provide a tool for understanding patterns in ecological communities. However, apparent network patterns observed in real-world systems may be influenced by a skewed species abundance distribution (SAD), which obscures the differences between biology- and chance-based drivers of network patterns. This dissertation addresses that issue using a four-year experimental network in which the relative abundance of 17 plant species is even, and from which 8,054 specimens of 104 bee species were collected. Patterns observed in that even-SAD field experiment were compared with null models, to answer the following questions: (1) Can the apparent nested pattern of plant – pollinator networks inform pollinator conservation? (2) Does the number of plant species partners and the number of plant individual partners equally influence the effect of losing plant species from networks, and does this depend on the plant SAD? (3) Is apparent year-to-year variation in interactions driven by biology or by chance, and does this depend on the bee species abundance? This research shows that (1) the abundance of rare and common bee species were uncorrelated across plant species, suggesting that their divergent preferences would require a targeted, not a general, choice of plant species for conservation. However, their preferences were positively correlated

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when the even plant SAD data were subsampled to simulate a skewed plant SAD network, but only when the most attractive plant species was simulated to be the most abundant. (2) Plant species' differential importance in the process of network disassembly is driven more by number of partner species than partner individuals, but only when the plant SAD is even. Almost no difference between species- and individualbased plant species loss was observed in the skewed network, because subsampling to skew the SAD removed many rare species and caused partner species and individuals to become more tightly correlated. (3) Expected annual dissimilarity in bee species' preferences decreases with bee abundance, suggesting an increased ability to detect nonrandom changes for common bees. The common bees differed significantly from that null expectation, suggesting that variation driven by biology, not by chance, is only detectable for common species.

IV. Acknowledgement

Thank you to the US Environmental Protection Agency (EPA) STAR Graduate Fellowship; the Kontos Family; Rutgers University Department of Ecology, Evolution, and Natural Resources Charlie Kontos Memorial Grant, Ted Stiles Award, and Buell Award; and the Garden Club of America for funding assistance. I am grateful to the staff of the Natural Resources Conservation Service (NRCS) Cape May Plant Materials Center for providing the site for the plots and on-the-ground assistance with plot setup and maintenance. Maria van Dyke collected the data in 2010. Thank you to Chava Weitzman, Jolie Dollar, Samantha Hauser, Chava Weitzman, Kayla Collins, Jasmeet Bawa and Kurtis Himmler for field and lab help. John S. Ascher identified all non-*Lasioglossum* bee specimens and Jason Gibbs identified all *Lasioglossum* bee specimens. Thank you to my lab group for providing feedback and support. Last but not least, thank you to my family and friends for supporting me through the process of creating this dissertation.

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I. Introduction

The study of pollination ecology has developed from its early focus on single-species interactions to a broader focus on entire plant and pollinator communities. Such community level inquiry allows ecologists to explore the ways in which community composition determines plant-pollinator interaction patterns and processes.

The patterns that are observed in plant – pollinator communities can be divided into those driven by biology or those driven by chance. Biological factors that influence interaction patterns include the attractiveness of plants (e.g., pollen or nectar production or flower quantity; Potts et al. 2003; Junker et al. 2013) or by pollinators' behavioral (e.g., preference, Tuell et al. 2008, Russo et al. 2013) or morphological (e.g., proboscis length, Stang et al. 2005) characteristics. Chance-based factors include plant or pollinator abundance, which could fluctuate within or across years (Stang et al. 2007, Blüthgen 2010). Chance could influence the detection of interactions, such that abundant species are encountered and sampled more than rare species, and those abundance-based processes themselves could drive a majority of observed community patterns (Stang et al. 2007; Vazquez et al. 2009). Both biology and chance-based patterns could singly or together influence plant – pollinator community patterns.

However, there exists a universal, underlying phenomenon of ecological communities that could influence the ability to distinguish biology and chance. That phenomenon is the species abundance distribution, or SAD. In most ecological communities, the SAD is skewed—a few species are abundant, and the majority are relatively rare (McGill et al. 2007). The skewed SAD could confound the differences between biology- and chance-based drivers of plant – pollinator interaction patterns in

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that the relative importance of abundant species for a given ecological process, and the redundancy of rare species for that process, may be driven by species traits that are separate from abundance (e.g. pollen or nectar rewards of plants), or by abundance itself—but when species abundances are skewed, it is impossible to empirically tease apart those patterns (Bluthgen et al. 2008; Bluthgen 2010).

One area of plant-pollinator community research in which biology and chance have been potentially confounded is networks. In plant-pollinator networks, particular patterns are measured and attributed to biological mechanisms. First, species are arranged on a continuum ranging from apparently specialized (few interaction partners on the local, community level) and apparently generalized (many interaction partner species) (Bascompte 2003). Network analysis of plant – pollinator communities shows that only a few species tend to be generalized, and many tend to be specialized. Second, species do not randomly interact—instead, the specialists tend to interact with a proper subset of the generalist species' partners. This pattern is referred to as 'nestedness' (Bascompte 2003). Network analysis could confound the differences between biology- and chance-based drivers of plant – pollinator interactions in that the apparent 'specialization asymmetry', and resulting pattern of interactions between generalists and specialists, may be driven largely by a frequency asymmetry of species (Bluthgen 2010). This means that by chance, the apparent generalists appear so because they are common, and the apparent specialists appear so because they are rare. Null model analyses of plant – pollinator networks acknowledge that differences in species abundances may be important drivers of interaction patterns (Stang et al. 2007, Vazquez et al. 2009), but to date very few network analyses use empirical methods to account for abundance asymmetries, and a

large majority of such analyses make inferences that could be confounded by chance.

This dissertation examines three specific areas of plant pollinator network research that may confound biology and chance. First, the nested interaction structure suggests that rare and common bee species may use, and be conserved by the apparent generalist plant species. But if a skewed SAD drives those patterns, then networks are only describing bees' differential use of plants, not their differential preference of plants (Johnson 1980). Thus using information from plant-pollinator networks in which the plant SAD is skewed may bias the information used to conserve and restore bee species. Second, network disassembly simulations that remove species of one trophic level (e.g. plants) and observe the effects on other species in the network, often conclude that the number of species partners of plants or pollinators determines those plants' or pollinators' importance in disassembly—that is, whether losing that species will drive rapid secondary extinctions of partners, or whether losing that species will have a very minor effect (Memmott et al. 2004, Kaiser-Bunbury et al. 2010). However, those simulations have not accounted for differential species abundances, and whether the effect of losing species of one trophic level may be influenced by a chance-based sampling effect. Third, studies that show that the occurrence of interactions between plants and pollinators varies across years acknowledge that species turnover may generate annual variation, but they do not account for changes in species abundance beyond any apparent changes in presence/absence (Petanidou et al. 2008, Alarcón et al. 2008, Burkle & Irwin 2009; Trojelsgaard et al. 2015). Furthermore, to the best of our knowledge no study has established a null model for the expected amount of interaction variation across years, and compared the observed variation to that null.

The goal of this dissertation is to separate the effects of biology from those of chance by using a four-year experimental plant-pollinator network in which the relative abundance of plant species is even, and in which plant species are evenly sampled, within and across years. Along with this field experimental data, three null models are established and compared to the field experiment. These analyses address the following questions: (1) Can plant – pollinator network research inform pollinator conservation? (2) How do species abundance distributions influence plant – pollinator network disassembly? (3) How do plant – pollinator networks change across time?

Chapter 1. Rare and ecosystem service-providing bee species do not prefer the same plant species, despite the predictions of network analysis

Abstract

In plant-pollinator networks, the rare or specialist species with few interaction partners tend to interact with common or generalist species which themselves have many partners, both common and rare. This nested network structure means that the common/generalist plants should host both rare and common pollinator species. However, whether rare and common bees within the same network prefer the same plants, and whether this extends to regionally rare bee species in need of protection, and the common generalist bee species that provide ecosystem services to crops, has not been tested. We tested this prediction using a field experiment consisting of 102 monospecific plots of 17 plant species from which we collected bee pollinators over four years. All plant species were present at the same abundance, thus removing any sampling effects caused by a skewed species abundance distribution for plants, and allowing us to directly measure pollinator preference. We also used a large independent data set to identify bee species that are regionally rare, or that are important providers of ecosystem services (crop pollinators: hereafter ES bees). Both rare and common bees had strong, significant preferences for certain plant species, but neither locally rare and common bees nor the regionally rare and ES bees preferred the same plants. To explore the mechanism behind our results, we conducted a simulation study that resampled from our even plant species abundance distribution to create a skewed distribution similar to the natural communities upon which our original prediction from network analysis is based. This simulation re-created the results predicted from network analysis, that locally rare and common bees preferred the

same plants, but only when the most bee-attractive plant species were chosen to be the most abundant species. Our results suggest that the nested network structure found by many network analyses conducted on natural communities is attributable to sampling effects driven by the highly skewed species abundance distributions of the plants from which the pollinators are collected, combined with high pollinator attractiveness of the most abundant plant. **Synthesis and applications.** We show that two conservation target taxa, regionally rare bee species and bee species that provide ecosystem services to crops, prefer different plant species. We did not find support for the prediction of network analysis that the rare and common species would visit the same plants; rather that prediction appears to result from the methods of network analysis, which generally assess use and not preference. Pollinator habitat restoration should be based upon preference and our results suggest that a mix of plants will best support conservation target taxa.

INTRODUCTION

It is important to consider both rare and common species in conservation management. Rare species protection is easy to justify based on either extinction risk (Mace *et al.* 2008) or in some cases, the aggregate importance of rare species for ecosystem function (Lyons *et al.* 2005; Mouillot *et al.* 2013). However, common species are also important to protect if they are declining (Gaston 2010) or important providers of ecosystem services (Kleijn *et al. In review*). Both approaches are highly suitable for pollinators, as many pollinator species are rare (Nieto 2014), and pollinators are also important providers of the ecosystem service of crop pollination (Kleijn *et al. In review*). In pollinator conservation, the main management action is establishing patches or hedgerows of flowering plants (Menz *et al.* 2011). Thus it would be useful to know if rare species and common, ecosystem service-providing (ES) pollinators can be efficiently restored using the same plants.

In order for pollination conservation plantings to be most effective, the choice of plants should account for pollinators' actual preferences. Preference should be as measured in a choice experiment in which the number of plant individuals, or the area planted, is standardized across plant species (Comba et al. 1999; Corbet et al. 2001; Tuell et al. 2008; Russo et al. 2013). Thus in a choice experiment, when a pollinator shows a preference it could be based on variation among plant species in flower quantity (i.e. number of flowers per individual plant, or per standardized area; (Thompson 2001; Glaettli & Barrett 2008) or quality (pollen, nectar, or other floral traits (Potts et al. 2003; Junker *et al.* 2013), but not on differences among plant species in the number of plant individuals or area covered. However, apparent pollinator preferences as measured in natural communities are more reflective of pollinators' differential use of, not preference for, plant species, because pollinator specimens are sampled from natural plant communities with highly skewed species abundance distributions (Johnson 1980; McGill et al. 2007; Bosch et al. 2009). Thus in natural communities more pollinator species might use (be collected from) the abundant plant species, simply due to plant abundance and sampling effects (Bosch et al. 2009; Blüthgen 2010). For the same reason, both the common and the rare bee species might be collected from the abundant plant species because these plants are better sampled, i.e., by chance alone (Bosch *et al.* 2009; Blüthgen 2010).

For the same reason, apparent interaction patterns among rare and common species in natural networks could be caused, at least in part, by a skewed speciesabundance distribution (SAD). For example, in networks, the number of partners a species has in a network corresponds to its abundance, so that species with few partners (often termed specialists in the network literature) tend to be rare species, and species with many partners (often termed generalists) tend to be common (Ashworth 2004; Dorado et al. 2010). A related finding is that mutualistic networks tend to be nested and asymmetrical, such that the rare species are observed interacting with common species, and common species with both common and rare species (Bascompte et al. 2003; Bascompte & Jordano 2007; Blüthgen et al. 2008). (Vázquez & Aizen 2004; Blüthgen et al. 2008). Thus based on the findings from natural network studies, one would expect to find rare and common pollinators using the same, common plant species. However, if this result is being driven largely by sampling effects (Blüthgen et al. 2008; Blüthgen 2010), as suggested by some null model analyses that allow species to interact in proportion to their abundances (Vazquez, Chacoff & Cagnolo 2009; Ibanez 2012; Vizentin-Bugoni, Maruyama & Sazima 2014), then this finding should not be used in conservation practice. Alternatively, as also suggested by some null model analyses of data from natural systems (Vazquez, Chacoff & Cagnolo 2009; Olito & Fox 2014), if particular plant species offer better pollen and/or nectar rewards and for this reason are more attractive to both common and rare pollinator species, then identifying such plants could significantly improve the ecological and economic effectiveness of pollinator restoration measures.

From a conservation perspective, two types of pollinators are of particular interest in pollinator restoration: the regionally rare species, which will be at greater at risk of extinction, and the species that provide ecosystem services to crops, which tend to be regionally common species ((Vázquez, Morris & Jordano 2005; Kleijn *et al. In review*). If the same species that are rare regionally are also rare in a given network, and the same species that are regionally common and thus are ES providers are also common in the network, then the above arguments about plant preference versus use for rare and common species within the network could apply to the regionally rare and ES conservation targets as well. However, even if this is not the case, it is of interest to determine what plant species both types of conservation targets (regionally rare and regionally ES) prefer, and whether they prefer the same plants.

In this paper we use an experimental plant-pollinator network in which each of 17 native plant species is represented by the same number of individuals (i.e., the SAD is perfectly even), and a subsampled version of that data that represents a skewed-SAD plant-pollinator network, to answer the following questions: (i) Do bee species that are rare and common within the network prefer the same plant species within an experimentally even plant community? (ii) Do these same rare and common bee species use the same plant species in a subsampled version of our network, in which the plant SAD is skewed similarly to natural systems? We then use independent datasets to identify the regionally rare and key ES-providing species, and ask (iii) Do bee species that are regionally rare, and bee species that regionally are important providers of ecosystem services, prefer the same plant species?

MATERIALS AND METHODS

NETWORK DATA: THE FIELD EXPERIMENT

In the fall of 2009, we established an experimental array of 17 native, perennial plant species (Table 1) located in a former old field near Cape May, New Jersey, USA (39.07 N, -74.46 W). Plants were established in 1 m², single-species plots each of which contained 9 mature individual plants. We used a randomized block design with six replicate plots per species for a total of 102 plots. We used plant species that we expected to be attractive to native pollinators, based on our previous field studies in our study region, and which additionally were suitable for use in pollinator habitat restorations (e.g, commercially available, easily propagated, but not considered weeds). We maintained the plots throughout the four-year experiment by weeding, watering, and replacing plants that died over the winter. Given that the bee species in our study forage over 100 to 10,000 meters (Greenleaf *et al.* 2007) and our entire study array was 18 x 57 meters, our design creates a choice experiment for foraging bees.

From May to September in each of 2010-2013, we netted all flower-visiting bees to each plot on three separate days during peak bloom. On each of those three days, each plot was sampled twice per day, during separate ten-minute morning and afternoon periods. Within a single day, the order of plot sampling was randomized. We limited data collection to times when weather was sunny or partly cloudy, temperatures $\geq 16^{\circ}$ C, and mean wind speed was ≤ 3 m/s, with rare exceptions (less than 1% of sampling events occurred at greater wind speeds). All specimens were fully curated, barcoded, and identified to species level by two professional taxonomists (J. Ascher, National University of Singapore, and JG).

We measured floral abundance once per year in each plot by counting the number of open flowers during the peak bloom of each plot. We calculated average area per flower by photographing ten representative flowers per species with a ruler alongside the flower for scaling purposes, and used ImageJ software (Schneider, Rasband & Eliceiri 2012) to measure the pixels per flower. We converted pixels per flower into mm², our measure of floral area, and calculated the average mm² across the ten flowers. Floral area per plot/year was then calculated by multiplying the total number of open flowers in that plot and year by the mean area per flower.

We defined our list of rare bees in the network based on the distribution of individual bees per species collected in the field experiment. We defined the rare bees as those represented by ten or fewer specimens across all four years of the study (Supplementary Figure 1), a threshold we determined based on a natural break in the distribution of species abundances (Supplementary Figure 1). We hereon refer to this group as the locally rare bees.

We defined our list of common bees in the network based on the distribution of individual bees per species collected in the field experiment. We defined the common bees as the minimum set required to contribute 80% of specimen records. This threshold includes species represented by 187 or more specimens, which is a natural break in the distribution of species abundances. We hereon refer to this group as the locally common bees.

DATA ON REGIONALLY RARE AND ECOSYSTEM SERVICE-PROVIDING SPECIES

We used existing data sets from RW's laboratory to determine which bee species are regionally rare, and which are regional ES providers, where regional is defined as within the state of New Jersey, USA. We determined which species were regionally rare using a database of 13,738 wild bee specimens of 262 species which we, or other members of our laboratory group, collected between 2003 and 2013 from a total of 192 sites located in natural (e.g. forests, meadows) or semi-natural (e.g. field margins) habitats. All specimens were collected using either hand nets or pan traps. Further details of the studies and data collection can be found in (Winfree, Griswold & Kremen 2007; Winfree *et al.* 2014), and detailed information on data obtained from each study found in Supplementary Table 1. We hereon refer to this database as the regional rarity database.

We determined which species were regionally important to ES using a database of 7266 wild bee specimens collected between 2003 and 2013 from watermelon, cranberry, blueberry, and apple farms in the state of New Jersey. Specimens were collected from flowers of crop plants using hand nets. Further details of the studies and data collection can be found in published sources for cranberry (Cariveau *et al.* 2013; Benjamin, Reilly & Winfree 2014), blueberry (Benjamin, Reilly & Winfree 2014), and watermelon (Cariveau *et al.* 2013), with detailed information on data obtained from each study found in Supplementary Table 1 in this manuscript. We hereon refer to this database as the regional crop database.

ANALYSIS METHODS

DO BEE SPECIES THAT ARE RARE AND COMMON AS MEASURED WITHIN THE NETWORK VISIT THE SAME PLANT SPECIES WITHIN AN EXPERIMENTALLY EVEN PLANT COMMUNITY?

To compare plant species preference by locally rare or common bees, we used a generalized linear model (GLM) with negative binomial error structure with the outcome variable being the abundance of rare or common bees summed across the three collection days of each plot (two separate models). We included plant species and year as fixed effects, and floral area per plot, measured during one of the three collection days, as a covariate. This analysis was performed in R (Version 3.0.3, R Core Team 2013) using the glm.nb() function in the MASS package (Venables & Ripley 2002).

To determine whether plant species that attracted a high (or low) abundance of rare bees also attracted a high (or low) abundance of common bees, we performed a Spearman rank correlation between the model estimates for rare and for common bees, with n=17 plant species as the replicate. Since the Spearman correlation is rank-order, it allows us to compare rare and common bee preferences even though they are not normally distributed.

DO THESE SAME RARE AND COMMON BEE SPECIES USE THE SAME PLANT SPECIES IN A SUBSAMPLED VERSION OF OUR NETWORK, IN WHICH THE PLANT SAD IS SKEWED SIMILARLY TO NATURAL SYSTEMS? To test whether our experimental treatment of creating an even plant SAD influenced the correlation between rare and common bee abundance across plants, we created a null model using that even-SAD data, representing the sampling intensity of the skewed-SAD plant-pollinator networks typically studied in natural systems. To create this null model, we subsampled the data in our field experiment as follows. First, we created a matrix of all possible sampling events for each of the 17 plant species. This included each unique ten-minute morning or afternoon period for each plot of each plant species, for a total of 144 sampling events per plant species. Those sampling events were in the matrix rows, and the abundance of each pollinator species in the columns. Sampling events that resulted in no pollinators were accounted for in rows with all zeros. We then excluded a certain number of samples per plant species such that the resulting plant species abundance distribution mimicked the log-normal distribution commonly observed in natural systems. To accomplish this, we pulled 17 values from a log-normal distribution and scaled these values such that the minimum equaled 1, representing the fewest possible sampling events per plant species, and the maximum equaled 144, representing the most possible sampling events per plant species. Each of the 17 plant species was assigned one of the 17 values, which described the number of sampling events to be preserved for that plant species. The values were ranked from highest to lowest, and assigned to plants in three different ways. First, we assigned the resampling values to plant species randomly. Second, we ranked plant species based on their attractiveness to bees in our even-SAD dataset (thus representing their actual preference by bees) and assigned the largest resampled plant abundance values to the most bee-attractive plant species. Third, we assigned the largest resampled plant abundance values to the least beeattractive plant species. We repeated each process 1000 times, and then averaged the results of those runs together. The final result is three simulated plant-pollinator networks, in which the species abundance distribution is skewed randomly with respect to plant species, or skewed in favor of either the most- or the least-attractive plant species.

To determine whether the congruence of rare and ES bees changes as a result of skewing the plant SAD, we repeated our GLM with negative binomial error structure and Spearman's rank correlations as described above for the even-SAD data, but using only the bee specimen data from the sampling events for each plant species chosen in the skewed-SAD simulation. Thus we determined whether plant species that have a high (or low) abundance of rare bees also have a high (or low) abundance of common bees when plants are not evenly sampled.

DO BEE SPECIES THAT ARE REGIONALLY RARE, AND BEE SPECIES THAT REGIONALLY ARE IMPORTANT PROVIDERS OF ECOSYSTEM SERVICES, PREFER THE SAME PLANT SPECIES, AND WHICH PLANT SPECIES DO THEY PREFER?

WHICH BEE SPECIES ARE REGIONALLY RARE?

Because wild bees tend to have highly variable population sizes on the local scale (Williams, Minckley & Silveira 2001), we chose to classify regionally rare bees based not on local abundances across sites, but on the number of sites that each species occupied in our study region, following the methods of (Mouillot *et al.* 2013). We

defined the rare species as those present at only 1 or 2 sites out of our total 78 sites, a threshold we determined based on a natural break in the distribution of site occupancies (Supplementary Figure 2). We hereon refer to this group as the regionally rare bees.

WHICH BEE SPECIES ARE THE MOST ABUNDANT ECOSYSTEM SERVICE PROVIDERS TO CROPS IN OUR REGION?

We defined our regional list of abundant, key ES providing bee species to crops as follows. Within each single crop, we ranked bee species by their proportional abundance. We then determined the minimum set of species responsible for a majority of visits to that crop, defined as 80% of crop flower visits (i.e., 80% of collected specimens; Supplementary Figure 3). Previous research has shown that visitation frequencies are positively associated with the amount of pollination services delivered (Vázquez, Morris & Jordano 2005); even if an individual rare bee is highly effective on a per-flower visit basis, the value of that species to ecosystem service provision is minimal, simply because that pollinator species is represented by very few individuals (Kleijn *et al. In review*). We combined the list of most abundant bee species within each crop into one across-crops summary list; we hereon refer to this across-crops list as the regionally ES bees.

DO BEE SPECIES THAT ARE REGIONALLY RARE, AND BEE SPECIES THAT REGIONALLY ARE IMPORTANT PROVIDERS OF ECOSYSTEM SERVICES, PREFER THE SAME PLANT SPECIES, AND WHICH PLANT SPECIES DO THEY PREFER? We used the data from our field experiment in a parallel way to section (i) above, but instead of determining the congruence between locally (network-level) rare and common species, we repeated those analyses for the regionally rare or ES bees.

We additionally determined which plant species the regionally rare or ES bees preferred in order to provide plant species recommendations for pollinator restoration. To determine which plant species attracted a significantly higher abundance of rare or ES bees, we compared each plant species' rare or ES bee abundance to the mean estimate of rare or ES bees using pairwise contrasts in R package multcomp (Hothorn, Bretz & Westfall 2008). We defined a plant species that was preferred by rare or ES bees as one with significantly higher abundance compared to the mean.

We used Spearman's rank correlation to determine the congruence between a species' abundance in our network field experiment and its regional abundance. We did this in two separate analyses: one for the bee species that were found in both the field experiment and the regional rarity database (n=63 species), and a second test for the bee species that were found in both the field experiment and the regional rarity database (n=42 species).

RESULTS

The network field experiment data set over four years and 2,448 per-plot collection events, totaling 409 hours of data collection, consists of 8,054 specimens of 104 species. Of these, 46 species were represented by ten or fewer individuals; we designated these the rare species in our network. Ten species collectively represented 80% of individual flower visits; these species were represented by 187 or more individuals. We designated these as the common species in our network.

The regional rarity dataset consists of 13,090 specimens of 237 wild bee species. The regional ecosystem services dataset, combined across the four crops, consists of 7,267 individual bee specimens of 127 species.

Ninety-four species were collected in only 1 or 2 sites; we defined these 94 as the regionally rare bee species (Supplementary Figure 2; Supplementary Table 1). These species represent approximately a third of all native bee species collected in the study region, but account for only 3% of the total individual bee specimens. 17 of these species were collected in our network field experiment, for a total of 449 individual bee specimens. The correlation between the local- and regional-level rank abundances of bee species that were present in both our field experiment and in our rarity dataset was not significant (rho=0.21, p=0.09). Twenty-four species (approximately 19% of the 127 cropvisiting species) collectively provided 80% of visits to the four crop plants; we defined those 24 bee species as the key ecosystem service providers for the study region (Supplementary Table 3). Ten of the 24 ES bee species were recorded in our network field experiment, for a total of 2,956 individual bee specimens. The correlation between the network- and regional-level rank abundances of the bee species that were present in both our field experiment and our crop dataset was low, but significant (rho=0.30, p=0.05).

There was no overlap in species identity between rare and ES species.

DO BEE SPECIES THAT ARE RARE AND COMMON AS MEASURED WITHIN THE NETWORK VISIT THE SAME PLANT SPECIES WITHIN AN EXPERIMENTALLY EVEN PLANT COMMUNITY?

Plant species varied significantly in the abundance of rare and common bees (Table 1), and floral abundance was positively associated with both rare and common bee abundance ($p \le 0.01$). However, overall and across all 17 plant species, model estimates for rare and common bee species were not correlated (Figure 1; rho = 0.06; p = 0.8), indicating that the two groups did not prefer the same plant species.

DO THESE SAME RARE AND COMMON BEE SPECIES USE THE SAME PLANT SPECIES IN A SUBSAMPLED VERSION OF OUR NETWORK, IN WHICH THE PLANT SAD IS SKEWED SIMILARLY TO NATURAL SYSTEMS?

The lack of correlation between rare and common bee preferences did not change as a result of our manipulation of the species abundance distribution of plants when subsampling order was random with respect to plant species identity (Table 1, Rho=0.09, p=0.7), or when plant species were subsampled in increasing order of bee abundance (i.e., less attractive plant species retained more samples, Table 1, Rho=0.009, p=0.97). However, when plant species were subsampled such that more attractive plants retained more samples, the abundance of rare and ES bees was positively correlated across plants (Table 1, Rho=0.47, p=0.05).

DO BEE SPECIES THAT ARE REGIONALLY RARE, AND BEE SPECIES THAT REGIONALLY ARE IMPORTANT PROVIDERS OF ECOSYSTEM SERVICES,

PREFER THE SAME PLANT SPECIES, AND WHICH PLANT SPECIES DO THEY PREFER?

The abundance of regionally rare and ES bees varied significantly across plant species (Table 1). Five plant species had significantly higher rare bee abundance compared to the mean. In descending order of preference, those species were *Penstemon hirsutus*, *Agastache scrophulariifolia, Lobelia siphilitica, Oligoneuron rigidum,* and *Eupatoriadelphus maculatus*. Five plant species also had significantly higher ES bee abundance compared to the mean. In descending order of preference, those species were *Pycnanthemum tenuifolium, Asclepias incarnata, Lobelia siphilitica, Asclepias tuberosa,* and *Veronicastrum virginicum.* In all models, floral abundance was positively associated with both rare and ES bee abundance ($p \le 0.01$).

One plant species, *Lobelia siphilitica*, was preferred by both rare and ES bees. However, overall and across all 17 plant species, model estimates for rare and ES bee species were not correlated (Figure 2; Table 2; rho = 0.30; p = 0.20), indicating that the two groups did not prefer the same plant species. Sensitivity analysis on different threshold proportions of crop visits for defining ES bees did not affect this result (SI Table 1).

DISCUSSION

Plant-pollinator networks typically have a nested interaction structure, in which the partners of specialized species are a proper subset of the partners of generalized species (Bascompte *et al.* 2003; Bascompte & Jordano 2007; Blüthgen *et al.* 2008). This network structure suggests that rare and common bee species in the network should use the same

plants; thus it may be possible to conserve both rare and common, ecosystem serviceproviding bee species with the same plant species. However, the skewed relative abundances of species in natural systems means that networks do not allow for the separation of pollinators' differential use of plant species from their actual preferences for particular plant species. We used a plant choice experiment to test whether rare and common bee species do in fact prefer (not just use) the same plant species. We found that while rare and common bee species each preferred particular plant species, the differences among plant species in the abundance of rare bees was not correlated with the differences among plant species in the abundance of common bees, indicating that these two bee species groups do not have the same preferences (Figure 1). However, we did find that rare and common bee preferences were positively correlated when we manipulated our experimental, even-species abundance distribution data set to approximate a real-life skewed species abundance distribution, which is the norm in nature (McGill et al 2007), but only when the real-life most bee-attractive plants were chosen as the most abundant plants.

In separate analyses, we identified the bee species that are regionally rare or regionally abundant crop pollinators, and determined whether those two groups preferred the same plants in our field experiment. Those results allowed us to recommend particular plants for species of actual conservation concern on a regional level. However, as was the case for the locally rare and common bees, the regionally rare or ES-providing bee species did not prefer the same plants. Together, our results suggest that the convergent plant preferences of rare and common pollinator species in real-life, skewedspecies abundance distribution plant-pollinator networks is a composite of plant species' differential abundance and attractiveness, and this pattern is related to nestedness, but not explained by it entirely.

One important contribution of our study is that our field experimental design allowed us to empirically tease apart a plant species' relative abundance from its attractiveness to pollinators. A plant species could attract an abundance of pollinators either because it is itself abundant, and thus a convenient foraging choice (Carvalheiro *et al.* 2014), or because it is attractive relative to other plant species for some reason apart from the abundance of individual plants (Waser *et al.* 1996; Chamberlain *et al.* 2014). Previous studies have used models (Vazquez *et al.* 2007; Vazquez *et al.* 2009; Olito & Fox 2014) or empirical data (Dáttilo, Guimarães & Izzo 2013) to explore the influence of species abundance on such observed mutualistic interaction patterns. However, we are not aware of any plant-pollinator network studies that empirically control plant relative abundance to test network predictions. We not only achieve this in our four-year field experiment, but we also then manipulate our dataset to compare it to the skewed-SAD systems common in nature, thus enabling us to tease apart the abundance- and nonabundance-based drivers of plant-pollinator interactions.

The lack of correlation among rare and common bee species appears to be driven by two factors that vary across plant species and that can be categorized into either abundance or attractiveness factors. First, the relative abundance of individual plants, which is what we controlled in our field study and manipulated in our simulation, strongly determined the results of the correlation. When plant relative abundance was controlled in our experiment, rare and common bees did not show the same preferences. Likewise, random subsampling across plants produced inconclusive results, because the results were strongly determined by the order in which plants were assigned samples: we found a positive correlation between rare and ES bee preferences when plant species were subsampled such that more attractive plant species retained more samples (thus retaining their relatively high bee abundance) and less attractive plant species retained fewer samples. When we reversed the order of subsampling such that less attractive plants retained more samples, there was no correlation between rare and ES bee preferences. This suggests that relative abundance may influence much of the apparent network patterns (nestedness and asymmetry) that suggest rare and common bees should visit the same plants, but that abundance itself does not explain these patterns; otherwise we would see rare and common bee species converging on the same plants regardless of the direction in which the SAD was skewed.

The second factor that could have influenced the lack of correlation among rare and common species in the field study is an unmeasured, non-experimentally controlled "attractiveness factor". In our abundance-controlled study, we show that both rare and ES species have preferences for particular plant species. Because all plant species were equally sampled, any variation in pollinator abundance was presumably caused by variation in plant traits that are separate from the amount of area covered by individual plant species (Junker *et al.* 2013), such as floral area (ref), reflectance (Campbell *et al.* 2010; Junker *et al.* 2013), scent (Raguso 2008) or the quantity and quality of pollen and/or nectar (Potts *et al.* 2003). The fact that particular bee species were significantly more abundant on particular plants when all plant species were equally available suggests that some combination of those attractiveness components varied across plants and influenced our results. Furthermore, any combination of these components can become more prominent over time as plant species establish, because some plant species will fill out their plots more than others. Thus while our original measure of pollinator preference was based on number of pollinators collected from the nine plant individuals we planted in the beginning of the study, our ultimate measure of preference, which is arguably most parallel to the way that pollinator restorations are established, was based on number of pollinators collected per unit area (meter squared), which was 'filled out' differently by plant species. Thus our field experiment allowed us to directly measure the attractiveness of plants that is not due to plant abundance, but the number of individual plants and flowers per plant species ultimately did vary due to differences in establishment patterns. The significance of floral area in our models suggests that this variation influenced their attractiveness, but the fact that we found significant differences among plant species even with floral area in the model suggests that floral area per se is only one potential attractiveness factor.

An additional important contribution of our study has to do with the relationship between a species' local abundance and its regional abundance, which has important implications for conservation. Many network studies infer a species' role in an interaction network (i.e., specialist or generalist), and thus its vulnerability to extinction, from its apparent niche breadth within that same network (Memmott, Waser & Price 2004; Vazquez *et al.* 2007; Vazquez, Chacoff & Cagnolo 2009; Plein *et al.* 2013). This is appropriate for understanding species dynamics on the local, network level, but in order to make regional conservation management decisions, it would be ideal to determine species' abundances using independently collected data, because those independent data would not be influenced by pollinators' localized patterns of plant species visitation (Vizentin-Bugoni, Maruyama & Sazima 2014). We found that there was no significant correlation between bee species' network rank abundance and regional rank abundance according to our non-crop, rarity database (Figure 1), and a significant, but low correlation between species' network and regional abundances on crops. Changing our definition of rare (or common) using regional, rather than network data, did not change the lack of correlation between these two groups' preferences as measured in our field experiment (data not shown).

Our study has a few important implications for pollinator conservation, an effort that has received significant attention and funding in recent years. First, while it is generally unknown for most systems which pollinator species are rare and which are the most important ecosystem service providers, our study uses multi-year datasets to define those species for our study region. Second, it is also unknown which plant species those rare or ES conservation target groups prefer. Our results enable us to identify those plant species based on rare and ES bees' preference for plants in an abundance-controlled field experiment. Furthermore, our results suggest that using data on pollinators' relative use of plant species from a skewed-SAD system may fail to identify the best plant species for restoring pollinators of regional conservation concern. These points are evidenced by the fact that our study was designed using plant species that were apparently preferred in their natural settings, but were not preferred, particularly by rare or ES species, when their relative abundance was empirically controlled. Our study lends support to other choice experiment studies that identify plant species for pollinator conservation (Comba et al. 1999; Corbet et al. 2001; Tuell et al. 2008; Russo et al. 2013), and expands on those results by incorporating independent analyses of which species require conservation on a regional scale. Our results show that landowners establishing pollinator habitat have a choice amongst several plant species, and while rare and ES bees may not have the same preference, landowners can choose among several attractive plant species to achieve multiple pollinator conservation goals within the same land management scheme.

TABLES

Table 1. Plant species used in network field experiment listed with unlogged model estimates comparing the abundance of rare or ES bees across plant species. Asterisks next to model estimates denote plant species with significantly higher abundance of regionally rare or ES bees compared to the mean estimate; these plants would be appropriate choices for restoring either bee species group. All plant species are native according to ITIS (http://itis.gov).

Plant species	Family		
		Rare	ES
Agastache scrophulariifolia	Lamiaceae	2.212*	0.937
Asclepias incarnata	Asclepiadaceae	1.129	4.754*
Asclepias tuberosa	Asclepiadaceae	0.856	4.003*
Eupatoriadelphus maculatus	Asteraceae	1.283*	2.482
Euthamia graminifolia	Asteraceae	0.040	1.391
Lobelia siphilitica	Lobeliaceae	1.929*	4.306*
Oligoneuron rigidum	Asteraceae	1.366*	2.065
Penstemon hirsutus	Scrophulariaceae	5.155*	0.496
Pycnanthemum tenuifolium	Lamiaceae	0.487	25.585*
Rudbeckia hirta	Asteraceae	0.071	0.232
Rudbeckia laciniata	Asteraceae	0.524	0.937
Solidago rugosa	Asteraceae	0.110	0.619
Symphyotrichum novae-angliae	Asteraceae	0.093	0.983
Vernonia noveboracensis	Asteraceae	0.536	0.406
Veronicastrum virginicum	Plantaginaceae	0.131	3.494*
Zizia aurea	Apiaceae	0.000	0.043

Crop	Plant species Cucumis melo. Citrullus	Number of species	Number of specimens	
Watermelon	lanatus	56		2368
Cranberry	Vaccinium macrocarpon	55		3666
Blueberry	Vaccinium corymbosum	42		1082
Apple	Malus domestica	34		151

Supplementary Table 1. Data sources for defining regional key crop-pollinating (ES) species.

Data sources for defining regionally rare bee species

		Number of	r of		
Number of species		specimens		Number of sites	
	137		1630	31	
	117		4172	16	
	191		3065	12	
	175		7003	19	
	Number of species	<i>Number of species</i> 137 117 191 175	Number of species Number of specimens 137 117 191 175	Number of species Number of specimens 137 1630 117 4172 191 3065 175 7003	

Supplementary Table 2. Regionally rare bee species

Andrena alleghaniensis Andrena asteris Andrena barbara Andrena brevipalpis Andrena commoda Andrena confederata Andrena fenningeri Andrena geranii Andrena hilaris Andrena hippotes Andrena hirticincta Andrena ilicis Andrena integra Andrena melanochroa Andrena nivalis Andrena placata Andrena rudbeckiae Andrena simplex Andrena uvulariae Andrena ziziae Anthidiellum notatum Anthophora plumipes Augochlorella persimilis Bombus auricomus *Bombus pensylvanicus* Coelioxys alternata *Coelioxys hunteri* Coelioxys immaculata Coelioxys moesta Coelioxys octodentata Coelioxys rufitarsis *Colletes bradleyi Colletes compactus* Colletes simulans Eucera hamata *Heriades carinata* Heriades leavitti *Heriades variolosus* Hoplitis pilosifrons Hoplitis simplex
Hoplitis spoliata *Hylaeus illinoisensis* Hylaeus sparsus Lasioglossum abanci Lasioglossum anomalum Lasioglossum atwoodi Lasioglossum cinctipes Lasioglossum ellisiae Lasioglossum georgeickworti Lasioglossum inconditum Lasioglossum katherineae Lasioglossum paradmirandum Lasioglossum rozeni Lasioglossum smilacinae Lasioglossum taylorae Lasioglossum truncatum Lasioglossum zephyrum Lasioglossum zonulum Lithurgus chrysurus Megachile addenda *Megachile campanulae* Megachile gemula Megachile petulans Megachile relativa Megachile texana Melissodes denticulata Melissodes trinodis Nomada bella *Nomada bethunei* Nomada luteola Nomada sayi Osmia albiventris Osmia collinsiae Osmia distincta Osmia inspergens Osmia lignaria Osmia sandhouseae Panurginus potentillae Perdita bradleyi Perdita octomaculata Pseudopanurgus

andrenoides Sphecodes atlantis Sphecodes carolinus Sphecodes coronus Sphecodes davisii Sphecodes dichrous Sphecodes fattigi Sphecodes minor Sphecodes pimpinellae Stelis labiata Trachusa dorsalis Triepeolus cressonii Triepeolus remigatus Supplementary Table 3. Key ecosystem service-providing (crop-pollinating) bee species from four crops in New Jersey. Shown here is a master list of the bee species that separately were the most abundant crop visitors that collectively provided 80% of crop visits.

Andrena vicina Andrena carlini Andrena nuda Ceratina calcarata dupla mikmaqi Andrena perplexa Bombus impatiens Andrena miserabilis Andrena morrisonella Andrena hippotes Bombus bimaculatus Bombus perplexus Osmia cornifrons Bombus griseocollis Melitta americana Andrena bradleyi *Xylocopa virginica* Colletes validus Habropoda laboriosa Lasioglossum imitatum Augochlora pura Lasioglossum versatum Melissodes bimaculata Halictus confusus Augochlorella aurata

FIGURES

Figure 1. Scatterplot of each plant species' rank of abundance of rare bees vs. that species' rank of abundance of common bees (each plant species represented by a single point; n=17 plant species). Spearman's rank correlations showed no relationship between rare and common bee abundance in the field experiment with even plant species abundance distribution (SAD) (rho = 0.06, p=0.80).



Figure 2. Scatterplot of each plant species' rank of abundance of rare bees vs. that species' rank of abundance of common bees, as measured by a simulation that skewed the plant species abundant distribution (SAD) either (a) randomly, (b) from most to least pollinator-attractive, or (c) from least to most pollinator-attractive. Each plant species represented by a single point; n=17 plant species. Spearman's rank correlations showed a positive relationship between rare and common bee abundance, but only when the plant SAD was skewed with the most pollinator-attractive plants simulated as the most abundant (b).





C Skewed Least to Most Attractive

Supplementary Figure 1. Bee species in the even plant-SAD field experiment has a skewed species abundance distribution. Rare species (left hand bar) were the 46 species represented by ten or fewer individuals out of a total 8,054 specimens of 104 species; common species were the ten species represented by 187 or more individuals.



Supplementary Figure 2. Frequency of bee species in New Jersey based on site occupancy from data collected by RW's lab group from 2003-2013. Rare species (left hand bar) were the 94 species out of a total of 237 species collected in 1 or 2 sites. 17 of these species were collected in our field experiment, for a total of 449 individuals.



Supplementary Figure 3. Frequency of bee species visiting cranberry flowers in New Jersey farms. ES species (circled) were the 5 species out of a total of 54 species that represented 80% of total collected specimens (200 specimens or greater). The key ES-providing bees for three other crops were defined using a parallel method and the key ES bee for each crop combined into a master list of 24 regional ES bee species. Details on data collection for cranberry found in Cariveau et al. (2013).



Chapter 2. Species abundance distributions and differences in plant attractiveness influence plant-pollinator network disassembly

Abstract

Many studies show that ecological networks are robust to the removal of specialists, but the loss of generalists causes secondary extinctions of generalist-dependent species. However, the skewed species abundance distribution (SAD) of ecological communities means that apparent differences in species' community disassembly roles may be driven by differential abundances and attractiveness, rather than by generalization per se. To explore this issue, we established a field experiment consisting of 102 monospecific plots of 17 plant species, with each plot standardized for relative abundance of plants. We collected bees visiting flowers to create an empirical network based on an even SAD for the plants. We then re-sampled from this even community to simulate a skewed SAD similar to natural communities. We compared secondary extinction rate from even or skewed-SAD plant communities by simulating plant extinctions either randomly, or in ascending or descending order of plants' partner richness (network 'degree') or partner abundance (network 'strength'). We use random species removal from either SAD network as a null by which to compare the robustness (area under the secondary extinction curve) of pollinator richness to our systematic species loss simulations. Because of our experimental design, disassembly patterns from the even-SAD network reflect differences in plant attractiveness to pollinators, but not differences in plant abundance that drive either actual interaction probability or sampling probability. Disassembly patterns from the skewed-SAD network thus reflect differences in plant abundance and attractiveness combined. In our four-year field experiment, we collected 7097 specimens of 83 bee species. After empirically removing variation in plant

abundance, we still found similar overall patterns to those reported in other plantpollinator network disassembly studies, in that relatively few particular species can either cause rapid disassembly, or can maintain network robustness. However, the relative importance of degree compared to strength depended on the underlying SAD, such that when the plant SAD was even, plant species degree deviated more from random loss, implying less robustness for pollinators than strength-based loss, but degree and strengthbased loss generated more similar robustness values when the plant SAD was skewed. This is likely due to the higher richness of rare pollinators in the even SAD network that were not detected when the SAD was skewed, and the tighter correlation between plant degree and strength that resulted from subsampling to create a skewed SAD. Thus in the skewed network the plant species with the most interactions also had the most partner species, and rare species that contribute to the robustness of pollinator richness are not detected. In summary, our study shows that plant species abundance and attractiveness together influence overall disassembly rate, but that the effect of relative abundance may conceal other plant species attributes (i.e. degree or strength) that themselves are strong drivers of secondary extinction patterns.

Introduction

Ecologists have long been interested in the influence of interspecific interactions on the dynamics of natural communities, with recent attention given to mutualistic interaction networks (Memmott, Waser & Price 2004; Aizen, Sabatino & Tylianakis 2012a; Campbell *et al.* 2012; Brodie *et al.* 2014). Understanding those dynamics informs both basic and applied ecology, given evidence that particular aspects of mutualistic interactions can influence the robustness of communities to species loss, which is

especially important in the face of global biodiversity loss (Tylianakis *et al.* 2008; Bascompte & Stouffer 2009). Of particular interest in mutualistic networks is the occurrence of secondary, or "linked" extinctions, by which the primary extinction of one species leads to the loss of its partner, thus compounding the intensity of the original extinction and contributing to the disassembly of the network as a whole (Brodie et al. 2014). In an effort to predict the causes and consequences of those secondary extinctions, ecologists have used models to simulate such mutualistic network disassembly. The basic approach to those simulations has been to systematically remove species under different hypothetical primary extinction scenarios, such as in ascending or descending order of species' generalization, and to observe the rate of decline of the remaining partner species in the network (Memmott, Waser & Price 2004; Kaiser-Bunbury et al. 2010; Campbell et al. 2012). The outcome variable of interest in such simulations is typically the proportion of partner species remaining after each primary extinction (Memmott et al. 2004; but see Kaiser-Bunbury et al. 2010). The assumption behind either approach to these simulations is that a species would become extinct from the interaction network (i.e. suffer from a local, "secondary" extinction) if all of its partner species were removed.

Generally speaking, these studies show that not all species are equally affected by (or influential to) mutualistic network disassembly, and this is largely due to three commonly observed traits of mutualistic networks. First, in networks, species are arranged along a continuum from specialized (few interaction partners) to generalized (many partners) in a pattern known as a skewed distribution of number of interactions per species, or *degree* distribution (Jordano, Bascompte & Olesen 2003; Bascompte & Jordano 2007). Second, the number of interaction partners that a species has is related to its abundance, such that the generalist species with the highest degree also have the highest number of total interactions, or *strength* (i.e., a generalist plant will tend to receive visits from many pollinator species and individuals) (Vázquez & Aizen 2003). Third, on average, the many specialist species tend to interact with the relatively few generalist species, and vice versa, in a pattern known as *specialization asymmetry* (Jordano, Bascompte & Olesen 2003; Bascompte & Jordano 2007). The skewed distributions of species degree and strength, along with specialization asymmetry, influence the simulated effect of losing any given species from networks, in that the most rapid rate of mutualistic network disassembly occurs following the removal of generalists, which also means that those species are more important for maintaining the robustness of the network as a whole (Memmott, Waser & Price 2004; Bascompte 2006; Kaiser-Bunbury et al. 2010). At the same time, the generalist species are also less sensitive than specialists are to the loss of their partners (Aizen, Sabatino & Tylianakis 2012a; Brodie et al. 2014). In contrast, networks are relatively robust to the loss of network specialists because those species have relatively few, usually generalist, partners (Memmott, Waser & Price 2004; Bascompte 2006; Kaiser-Bunbury et al. 2010).

However, the patterns observed in secondary extinction models may be biased by the underlying skewed species abundance distribution (SAD), which is a universal trait of ecological communities in which a few species are highly abundant and the majority are rare (McGill *et al.* 2007). The skewed SAD could generate any of the three network patterns described above via a sampling effect, through which species' relative probabilities of detection are related to their relative abundances (Nielsen & Bascompte 2007; Vazquez *et al.* 2007; Blüthgen *et al.* 2008; Vázquez *et al.* 2009; Blüthgen 2010). First, the skewed SAD will necessarily generate a skewed distribution of species strength within the network, unless species' participation in network interactions is disproportionate to their local abundances. Second, the skewed distribution of species degree could arise because a common species will by chance be detected interacting with more species, thus defining it as more generalized, and as having a higher 'degree', than a rare species. Third, because the distribution of a species' total interactions will be skewed across partner species in proportion to the local availability of each (Blüthgen, Menzel & Blüthgen 2006; Blüthgen *et al.* 2007), the skewed SAD could create specialization asymmetry because the infrequent detection of rare pollinator species will cause these species to preferentially associate with generalists in their few interactions (Bascompte *et al.* 2003; Bascompte 2006; Blüthgen 2010). Because these patterns are the result of a sampling effect across differentially abundant species, they would manifest regardless of whether the qualities that define species' true diet breadth or attractiveness to partners are actually related to abundance.

One way to determine the importance of species 'degree' and 'strength' on network disassembly, and whether the skewed SAD influences the importance of degree and strength, is to establish an expectation for the disassembly patterns that would emerge without any species-level differences in abundance or attractiveness to partners. Specifically, if all plant species were equally abundant and were chosen randomly by pollinators (thus making the simplistic assumption that there are no preference-driven pollinator choices based on plants' relative 'attractiveness'), then the shape of the secondary extinction curve would be identical regardless of the order of plant species loss. Thus in real-world plant-pollinator communities, any deviation from that null expectation could be caused by variation across species in either abundance or attractiveness. However, typical real-world systems lack standardization across species according to either of these factors, making it difficult to determine whether plant species relative abundance, rather than some other species-level trait (e.g. flower quality or quantity) that determines attractiveness is driving the observed secondary extinction patterns. Several previous studies have used models to explore the relative importance of species traits and abundances in driving interaction patterns among plants and pollinators (Stang, Klinkhamer & van der Meijden 2006; Santamaria & Rodriguez-Girones 2007; Stang, Klinkhamer & van der Meijden 2007; Stang et al. 2009; Vazquez et al. 2009; Bartomeus 2013). However, we are not aware of any research that has empirically separated the roles of plant abundance, and flower quantity and quality, in determining the number of pollinators attracted to the plant, and then incorporated both factors into secondary extinction models. Furthermore, despite evidence that plants that attract many pollinator individuals also tend to attract many pollinator species (Vázquez & Aizen 2003), we are unaware of any study that determines whether plant species degree (number of pollinator species partners) and strength (number individual pollinator visitors) have similar effects on the loss of pollinator richness as networks disassemble (but see Kaiser-Bunbury et al. 2010).

In this paper, we explore the potential influence of a skewed plant SAD on secondary extinction models by first collecting plant-pollinator network data from a field experiment in which the relative abundance of 17 plant species was controlled and in which sampling effort was equal across plant species. We then simulate pollinator community disassembly with plant species loss from that network in two ways: first using our network data from the even plant SAD, and second using a subsampled version of our network that approximates the skewed plant species abundance distribution of natural systems. We answer the following questions. (1) How does the species abundance distribution of plants influence the rate of pollination network disassembly when plants are lost according to number of pollinator species partners (i.e., species degree)? (2) How does the species abundance distribution of plants influence the rate of pollination network disassembly when plants are lost according to abundance of pollinator visitors (i.e., species strength)?

Materials and Methods

Datasets

Field experiment: the even plant SAD

In the fall of 2009, we established an experimental array of 17 native, perennial plant species in a former old field near Cape May, New Jersey, USA (39.07 N, -74.46 W). Plants were established in 1 m², single-species plots each of which contained 9 mature individual plants. We used a randomized block design with six replicate plots per species for a total of 102 plots. We used plant species that we expected to be attractive to native pollinators, based on our previous field studies in our study region, and which additionally were suitable for use in pollinator habitat restorations (e.g, commercially available, easily propagated, but not considered weeds). We maintained the plots throughout the four-year experiment by weeding, watering, and replacing plants that died

over the winter. Thus, given that the bee species in our study forage over 100 to 10,000 meters (Greenleaf *et al.* 2007) and our study array was 18 x 57 meters, our design creates a choice experiment for foraging bees.

From May to September in each of 2010-2013, we netted all flower-visiting bees to each plot on three separate days during peak bloom. On each of those three days, each plot was sampled twice per day, during separate ten-minute morning and afternoon periods. Within a single day, the order of plot sampling was randomized. We limited data collection to times when weather was sunny or partly cloudy, temperatures $\geq 16^{\circ}$ C, and mean wind speed was ≤ 3 m/s, with rare exceptions (less than 1% of sampling events occurred at greater wind speeds). All specimens were fully curated, barcoded, and identified to species level by two professional taxonomists (J.A. and Jason Gibbs, Michigan State University, USA).

Skewed-SAD simulation

To create a plant-pollinator network similar to those typically studied in natural systems, we subsampled the data our field experiment as follows. First, we created a matrix of all possible per-plot sampling events (each unique ten-minute morning or afternoon period for each plot of each plant species, for a total of 144 sampling events per plant species) in the matrix rows, and pollinator species in the columns. In the matrix cells, we recorded the abundance of each pollinator species that was detected during each sampling event. Sampling events that resulted in no pollinators were accounted for in rows with all zeros. We then excluded a certain number of samples per plant species such that the resulting plant species abundance distribution mimicked the log-normal distribution commonly

observed in natural systems. To accomplish this, we randomly sampled 17 values from a log-normal distribution and scaled these values such that the minimum equaled 1 and the maximum equaled 144. Each plant species was randomly assigned one of the 17 values, which described the number of sampling events to be preserved. The identity of the preserved sampling events was chosen randomly. We repeated the entire process described in this paragraph 1000 times, and then averaged the results of those runs together. The result is a simulated plant-pollinator network in which the species abundance distribution is skewed randomly with respect to plant species. We then combined all rows belonging to the same plant species, to transform the skewed- SAD network into the standard format with plant species as rows and pollinator species as columns. We also created an identical even-SAD network that included all sampling events for each plant species (i.e. it included all the field-collected data).

Analysis

Defining plant species degree and strength

First, we defined plant species' level of degree and strength for even and skewed networks separately. For the 1000 replicate skewed networks, plant species' levels of degree and strength varied depending on the quantity and identity of sampling events that were maintained in the process of creating the skewed plant SAD. Therefore, we defined plant species by degree and strength separately for every replicate skewed network. A plant species' degree, or network 'generalization', is the number of pollinator species that visit that plant species, obtained by calculating the number of non-zero cells in that plant species' row of the matrix. A plant species' strength is the number of individual

pollinators that visit it, and is obtained by calculating the row sums of the matrix. Clearly, degree and strength are not independent; for example, a plant species' degree cannot be greater than its strength. When degree and strength are highly correlated (Vazquez & Aizen 2003), we would expect each factor to affect the rate of disassembly in a similar way. However, degree and strength are not necessarily highly correlated, in which case they may have different effects on network disassembly. We determined the Spearman correlation coefficient ρ for the even-SAD network, and for all 1000 replicate skewed-SAD networks. We compared the even-SAD correlation ρ value to the distribution of ρ values belonging to the skewed-SAD networks.

Plant species removal simulations

To determine whether the plant SAD influenced the rate of secondary extinction when plant species were lost according to either degree or strength, we performed species removal simulations from our even- and skewed-SAD networks, and determined the rate of secondary pollinator extinctions that would occur from each network when plants were lost in ascending or descending order or degree or strength. To determine how the SAD of plants influenced the rate of disassembly when plants were lost according to degree, we simulated the loss of plant species in three alternative orders from even and skewed SAD networks separately. First, we removed plant species from highest to lowest degree, a hypothetical worst-case scenario in which the most-connected, network 'generalist' species are the first to go extinct from a network. Second, we removed plant species from lowest to highest degree, a hypothetical best-case scenario in which the least-connected network 'specialists' are the first to go extinct. Finally, we removed plant species randomly with respect to degree. Random species loss represents a null scenario by which to compare the two best-case (least linked species lost first) or worst-case (most linked species lost first) systematic species loss orders. We defined the rate of secondary extinction for all three extinction orders by quantifying the proportion of pollinator species remaining following the sequential removal of each plant species. We achieved this by removing one row in the plant-pollinator matrix (either at random or systematically based on plant species degree) and quantifying the loss of pollinators caused by that simulated plant species extinction by recalculating the proportion of columns that were greater than zero. We repeated this process for each plant species in the network, obtaining an extinction curve that represents the rate of pollinator loss caused by sequential plant species loss. Due to the process of subsampling the empirical data to create the skewed-SAD networks, not all pollinator species are present in all skewed networks. We measure secondary extinction as the proportion of pollinator species remaining to ensure that results are comparable across different skewed-SAD networks.

To determine whether removing plant species according to species strength had a similar effect as losing species according to degree, and whether this depended on the plant SAD, we repeated our species removal methods as above, except by defining species removal according to that level of interaction strength. We simulated the removal of plant species in the same three alternative orders as for the 'degree' method, i.e. from highest to lowest strength, lowest to highest strength, or randomly. Simulations for both 'degree' and 'strength' methods were carried out using the 'external' extinction order option in the second extinct function of R's bipartite package (Dormann, Gruber & Frund

2008), with the exception of the random species loss scenario, which was specified using the 'rand' extinction order option in second.extinct.

We quantified the importance of the SAD in determining the rate of secondary extinction obtained through simulations in two steps. First, for both even- and skewed networks, we determined the area beneath the random loss extinction curve and the area beneath each of the extinction curves representing the extreme scenarios in which plants were lost highest to lowest (HL) or lowest to highest (LH) based on degree or strength. These areas give robustness (R) values for each scenario, where higher robustness means that more bee richness is maintained despite losses of plant species. Effect sizes were then calculated as follows: $\Delta R = |R_{HL} - R_{random}| + |R_{LH} - R_{random}|$. The resulting value (ΔR) is the maximum total change in robustness that can be caused by any factor (e.g., degree, strength). In total, we obtained four ΔR values (even/skewed network by degree/strength loss order), and used these to quantify the differences between the effects of degree and strength on network disassembly, and to quantify whether the effects of degree and strength were different depending on the plant SAD.

Results

(1) How does the species abundance distribution of plants influence the rate of pollination network disassembly when plants are lost according to number of pollinator species partners (i.e., species degree)?

The correlation ρ between plant species strength and degree was 0.579 for the even-SAD network, compared with 0.806 (95% CI 0.638 to 0.955) in the skewed-SAD network (Supplementary Figure 2). Therefore, plant species ranks for degree and strength were

more highly correlated in the skewed-SAD network, and we expect the effects of degree and strength on robustness to be more similar in the skewed-SAD network.

Unsurprisingly, both even and skewed-SAD networks differed from the 'null' random loss expectation when plants were lost from highest to lowest degree, or from lowest to highest degree. As expected, for both even (Figure 1) and skewed (Figure 2) networks, the loss of plants from low to high degree generated a secondary extinction curve that suggested higher robustness to disassembly compared to random. In comparison, for both even and skewed networks, the loss of plants from highest to lowest degree led to a more rapid rate of secondary extinction, thus less robustness, than random. However, the differences from random species loss were slightly stronger in the skewed-SAD network, as indicated by ΔR values (skewed network: 6.33; even network: 4.06). Therefore, when plant abundance and attractiveness both vary across species (skewed-SAD networks), degree has a somewhat larger effect than when only attractiveness varies across species (even-SAD networks).

(2) How does the species abundance distribution of plants influence the rate of pollination network disassembly when plants are lost according to abundance of pollinator visitors (i.e., species strength)?

Parallel to our results for plants lost according to degree, both even and skewed-SAD networks differed from the random loss expectation when plants were lost in ascending or descending level of strength, such that networks were more robust to secondary extinctions when species were lost from lowest to highest strength, and less robust when species were lost from highest to lowest strength. However, the differences from random loss when plants were lost according to strength were markedly stronger in the skewed SAD network than in the even SAD network (ΔR of 5.42 for skewed and 1.76 for even). Therefore, when plant abundance and attractiveness both vary across species (skewed-SAD networks), strength has approximately three times the impact on robustness compared to when only attractiveness varies across species (even-SAD networks). Furthermore, in the skewed network, results for species loss according to strength were more similar to those according to degree (skewed network: ΔR of 6.33 for degree and 5.42 for strength). In comparison, losing species according to strength in the even SAD network differed much less from random than species loss according to degree (even network: ΔR of 4.06 for degree and 1.76 for strength), suggesting that 'degree' is more influential to secondary extinctions of pollinator richness than 'strength', but this was only the case when the SAD of plants was even.

Discussion

Decades of theoretical work (May 1973; McNaughton 1977; Pimm 1984; Albert, Jeong & Barabasi 2000; Ives & Carpenter 2007) and recent studies of mutualistic networks (Bascompte 2006; Aizen, Sabatino & Tylianakis 2012b; Campbell *et al.* 2012; Vieira, Cianciaruso & Almeida-Neto 2013; Rohr, Saavedra & Bascompte 2014) have found that particular attributes of interaction networks may contribute to network disassembly patterns. However, the underlying skewed species abundance distribution (SAD) of natural communities means that the effect of differential species abundances may be driving some of the reported disassembly patterns. Recent studies have used statistical approaches to investigate the effects of species abundances in mutualistic network properties (Stang, Klinkhamer & van der Meijden 2006; Stang, Klinkhamer & van der

Meijden 2007; Vazquez et al. 2007; Vazquez et al. 2009; Vazquez, Chacoff & Cagnolo 2009), but no previous work has compared the influence of species relative abundance with that of attractiveness on disassembly patterns. After removing variation in plant abundance (area) using a common garden experiment, we still found similar overall patterns to those reported in other plant-pollinator network disassembly studies, in that relatively few particular species can either cause rapid disassembly, or can maintain network robustness, depending on whether those species are lost or persist (Memmott, Waser & Price 2004; Kaiser-Bunbury et al. 2010; Brodie et al. 2014; Winfree et al. 2014). We demonstrate that the relative importance of degree compared to strength depended on the underlying SAD, such that plant species degree was more influential than strength when the plant SAD was even, but degree and strength-based loss generated more similar robustness values when the plant SAD was skewed. In summary, our study shows that plant species abundance and attractiveness together influence overall disassembly rate, but that the effect of relative abundance may conceal other plant species attributes (i.e. degree or strength) that themselves are strong drivers of secondary extinction patterns.

Our results contribute to previous studies of mutualistic network disassembly in two key ways. First, because we simulate plant species loss from a network in which the SAD of plants is even, we are able to approximate the disassembly pattern that is attributable to plant species' attractiveness to pollinators, rather than to a combination of attractiveness and plant species relative abundance. We consider 'attractiveness' of a plant species to be any trait related to floral resources that is not confounded by the relative abundance, or area covered, by each plant species. For example, the number of flowers, nectar, or pollen that a plant produces per unit area, or some other floral trait such as corolla depth or scent may all influence plant species' relative attractiveness (Junker *et al.* 2013). Thus our finding that robustness in the skewed SAD network differed more strongly from random than did robustness in the even SAD network suggests that both plant species attractiveness, as measured by plant species traits that vary within a controlled area, and relative abundance, as simulated by different numbers of sampling events across plant species, are driving the differences between plant species in their effects on secondary pollinator species loss. Using similar species loss scenarios, other network disassembly studies have shown that networks can lose many of the specialist species before networks disassemble, because the specialists are linked to relatively few species (Memmott, Waser & Price 2004; Kaiser-Bunbury *et al.* 2010; Aizen, Sabatino & Tylianakis 2012a; Campbell *et al.* 2012), but our result suggests that this robustness of networks to the loss of specialist species is at least in part influenced by an underlying SAD.

A second contribution of our study is our finding that the particular attributes used to order and remove plant species determined the overall disassembly pattern, such that extinction curves for plant loss according to degree and according to strength were not identical in their effects on secondary extinctions of pollinator species. As expected, in both networks, when plants were lost from highest to lowest degree (i.e., in descending order of number of partner species), pollinator richness tended to decline in a more linear fashion compared to plant losses that occurred randomly. Likewise, plants lost from lowest to highest degree also diverged from the pattern expected under random species loss, but in an opposite direction, such that pollinator richness declined less rapidly, and thus the network was more 'robust', when the least-connected species were lost first. (Figure 1, Figure 2). The results for plants lost according to strength were similar in that the loss of the species with the highest strength led to a more rapid rate of pollinator secondary extinctions than when plants with the lowest strength were lost first (Figure 3, Figure 4).

However, we found that the effects of degree and strength depended on the underlying SAD, such that secondary extinction patterns from the even SAD network were highly dependent on whether plants were lost according to degree or strength, but this difference was less pronounced in the skewed SAD network. The difference between plant loss according to degree vs. strength in the even SAD, but not the skewed SAD network, is intuitive given that previous plant-pollinator network studies, which take place in skewed SAD systems, have found positive correlations between the number of partner species (degree) and number of individual partners (strength) (Vázquez & Aizen 2003). In our even SAD network, there was a positive correlation between plant species degree and strength, but we found that this relationship became stronger when we subsampled our even SAD network to skew the plant SAD (Supplementary Figure 2).

A second, SAD-driven difference that could have influenced the different results for degree- versus strength-based loss has to do with sampling completeness. In our even-SAD network, many more rare species and more interactions between rare species and less attractive (in terms of strength) plants were present compared to in the skewed-SAD network (Supplementary Figure 1). Thus in the even-SAD network there were more interactions between rare pollinator and plant species, and the pollinator species in these interactions were especially vulnerable to secondary extinction because they likely had few plant partners. Because these pollinator species are rare, by definition they have few individuals and have little contribution to the overall 'strength' of the plants they visit; however, they contribute the same as common pollinators to 'degree'. Therefore these rare pollinators are more likely to go extinct quickly when high-degree plants are lost first compared to when high-strength plants are lost first. In contrast, subsampling plants to approximate a skewed SAD resulted in the 'loss' of many rare pollinator species from our evenly sampled data set. This suggests that the simulation of disassembly from network data in skewed SAD systems may fail to account for the loss of richness that occurs simply from the failure to detect it in the first place as a result of a sampling bias.

Our approach has a couple of caveats, which we believe could be considered in future analyses of plant-pollinator network disassembly. First, in adopting an established approach to mutualistic network disassembly (Memmott, Waser & Price 2004; Vieira & Almeida-Neto 2015) we make the simplifying assumption that pollinator species will go locally extinct following the removal of all of their host plants (LaBar *et al.* 2013). Because we do not incorporate species' variation in dependence on the mutualism (Vieira & Almeida-Neto 2015), or consider the probability that pollinators may switch host plants when their preferred host becomes unavailable (Kaiser-Bunbury *et al.* 2010; Abramson, Soto & Ona 2011; Ramos-Jiliberto *et al.* 2012; Valdovinos *et al.* 2013) our model lacks the dynamism that may exist in real-world plant pollinator networks. However, our intent was not to achieve ultimate biological realism. Instead, we aimed to isolate the importance of particular plant species attributes in disassembly by using an experimental dataset in which the abundance of plants was empirically, not just statistically, controlled. Furthermore, the previously reported probabilities of species switching hosts may themselves be influenced by a skewed SAD-driven sampling effect. Thus as far as we are concerned, there is no ideal biologically realistic model, but the results of the more dynamic approaches to network disassembly could be considered in the light of our findings about species abundance distributions.

A second caveat is that we assume subsampling from an even plant SAD network can simulate the network dynamics of a skewed plant SAD network. This presents the question of whether we would have found the same results if we had had two field experiments-one with a skewed SAD, and one with an even SAD that we subsampled from. However, in our simulations, we are not trying to exactly replicate a 'real world scenario.' Instead, our goal was to find out what would happen if we had sampled our plants differently, and subsampling from an originally even SAD is one such way to do that. While we could have in theory collected data from a skewed SAD system, and then used asymptotic richness estimators to approximate a more fully sampled plant community (Chacoff et al. 2012), we believe our approach is ideal because controlling abundance of plants in the field not only equalizes sampling effort, but also equalizes the probability with which each plant species will actually interact with pollinators, independent of whether anybody even collects data on the interaction (Vazquez, Chacoff & Cagnolo 2009). Thus any deviation from that equal interaction probability across plants will be caused by differences in plant species attractiveness that itself drives different preference-driven choices of pollinators. No amount of statistical extrapolation could determine the outcome if we had begun with a skewed SAD and estimated how pollinators would have responded under even-abundance conditions.

In conclusion, our findings on how a skewed SAD influences the relative importance of degree and strength network disassembly have important implications for the study of community stability and plant-pollinator networks. First, in real-world networks, a skewed SAD has a strong influence on observed network properties, such that particular species that contribute to network dynamics may be relatively over- or undersampled, and these sampling differences could influence the apparent drivers of disassembly. We replicated findings from other studies that show the importance of particular plant species for maintaining communities that are robust to species loss, but identity of those key species depended on the underlying plant SAD, and on the particular attributes (degree or strength) that were used to order and remove plant species. Figure 1. We subsampled the plant species in the even plant species abundance distribution (even SAD) data from our field experiment to approximate the sampling intensity across plant species that is typical of skewed-SAD natural systems. The entire light and dark shaded area of this plot represents the full 144 sampling events per plant species in the even-SAD field experiment. The dark shaded area represents the sampling events that were preserved in the skewed-SAD data, determined by randomly assigning scaled values from a log-normal distribution to plant species.



Figure 2. Results of simulated plant species loss scenarios demonstrate different effects of degree and strength on the rate of network disassembly, but the result depends on the species abundance distribution of plants. The X-axis of each panel represents the cumulative number of plant species removed in simulation; Y-axis represents the proportion of pollinator species remaining in the network following each sequential plant species loss. Top and bottom solid black lines represent plants lost from highest to lowest or lowest to highest degree or strength, respectively. Both systematic plant species loss scenarios are compared to random loss (dotted line). The effect size (deviation from random) of losing plant species according to a particular attribute (degree or strength) and from a particular SAD (even or skewed) are in the center of each plot.



Supplementary Figure 1. Interactions involving two rare species (i.e., a rare bee species and a rare or infrequently visited plant species; Rare Sp.-Rare Sp. Interactions) are less likely to be present in skewed-SAD networks than in even-SAD networks. Along the x-axis, we ranked interactions by the chance that they would occur if species interacted randomly (i.e. if abundance was the only factor structuring the network). Along the y-axis, we determined the proportion of skewed-SAD simulations that included each interaction and divided these proportions from one. We divided by one because, in the even-SAD network, all interactions were present by definition. Therefore, the y-axis can take on any value from 0 to 1.



Supplementary Figure 2. Histogram of Spearman's ρ values generated by the 1000 replicate skewed-SAD networks, with 95% confidence intervals (solid vertical lines). The dashed vertical line shows Spearman's ρ for the even-SAD network.



Chapter 3. Separating rewiring from chance in plant-pollinator network dynamics: a null model approach

Abstract

Recent multi-year studies of mutualistic interactions show that the identity and frequency of interactions between plants and pollinators change across years, because of a combination of species turnover and plasticity in interaction partners. Both biological mechanisms and chance could drive these patterns, but the relative importance of these factors has not been rigorously separated. We use a four-year field experiment, combined with a null model that controls for changes in species abundances, to determine the amount of Bray-Curtis dissimilarity in pollinators' preference for plant species that is due to chance, and the amount that is due to behavioral plasticity in preferences. In our field experiment, we collected 7097 specimens of 87 wild bee species, including in our analysis the 48 species that were collected in more than one year and which represented a range of relative abundances. Our null model predicted a general trend of rare bee species varying more across years (higher Bray-Curtis dissimilarities), and with higher 95% confidence intervals, than common bee species. Unsurprisingly, all 48 bee species' choice of plant partners varied somewhat across years. However, when empirical data were compared to the null, this variation was not significantly different from what would be expected by chance for 19 of the 48 bee species. Another 29 species rewired, meaning that they varied more across years than what would be expected by chance. However, rare species either did not differ from the null, or in a few cases varied slightly more across years than expected, while almost all of the common species varied more than the null. In summary, we establish, for the first time that we are aware, a null model for the

amount of interannual variation in plant-pollinator networks. We show that deviations from random are undetectable for rare species, and that common species display behavioral plasticity due to changing plant species preferences. Our null model approach could be used in other network studies that aim to compare the concordance of interaction matrices across time.

Introduction

Interactions between plants and pollinators influence the structure and functioning of ecological communities, with important contributions to global biodiversity (Bascompte & Jordano 2007). The plant-pollinator interactions that take place on a community level can be described as networks, in which the distribution of links (interactions) between nodes (species) is informative of natural community structure and dynamics (Ings et al. 2009). This network approach to studying plant-pollinator communities has been increasingly used in recent years (Bascompte & Jordano 2007). The few studies that have explored plant-pollinator networks over multiple years have found that they are highly dynamic over time, such that many differences arise when comparing the interactions that occur in one year to the interactions that occur in another (Petanidou et al. 2008; Dupont et al. 2009; Burkle & Alarcon 2011; Fang & Huang 2012). Consideration of these dynamics could influence our understanding of overall pollination network structure and the importance of particular species and interactions in networks, which in turn has implications for our understanding of ecological specialization and for the use of network analysis in conservation planning (Tylianakis et al. 2010; Blonder et al. 2012).

More specifically, studies of temporal variation in mutualistic networks show that species composition and pairwise interaction occurrences change across years, while

community-wide properties that define the network structure are temporally consistent (Alarcon, Waser & Ollerton 2008; Olesen *et al.* 2008; Petanidou *et al.* 2008; Dupont *et al.* 2009; Fang & Huang 2012). For example, a four-year network in the Mediterranean showed that only 53% of plant species, 21% of pollinator species, and 4.9% of plant-pollinator interactions were observed in all four years of the study (Petanidou *et al.* 2008). Similarly, a study of six qualitative European plant-pollinator networks ranging from 2 - 4 years in length showed similar variation, with high turnover occurring among the pollinators (27 - 56% of species present in all years) and interactions (11 - 22%) (Dupont *et al.* 2009). In these same studies, network-wide metrics such as nestedness, which is the tendency for species with few partners (often termed "specialists" in the network literature) to interact with a subset of the more connected species (often termed "generalists"), were consistent across years.

Both of the observed patterns in network dynamics—the consistency of networkwide metrics, and the variation in the identity of the species driving these metrics—could be caused by either biological features of networks, or by chance, but the relative importance of these two factors has not been rigorously separated. Thus far, plantpollinator network dynamics over time have been considered within the context of two biological factors that co-occur to generate temporal variation. The first is interaction plasticity, or "rewiring," a phenomenon that is observed when a plant-pollinator species pair is present from year to year, but does not interact every year (Petanidou *et al.* 2008; Dupont *et al.* 2009; Olesen, Stefanescu & Traveset 2011; Trøjelsgaard *et al.* 2015). Most previous studies have treated rewiring as a categorical phenomenon, such that an interaction is either present or absent; however, rewiring could also be considered as a quantitative process through which a given species pair interacts every year, but more frequently in some years than others (e.g. Alarcon et al. 2008; Burkle and Irwin 2009). Such rewiring presumably reflects the ability of species to adapt to changes in resource availability (Ramos-Jiliberto et al. 2012; Kaiser-Bunbury et al. 2010); and has been used to make inferences about species niche breadth (Petanidou et al. 2008).

However, network dynamics could also be influenced by chance. Although several studies acknowledge that species turnover influences the occurrence of interactions (i.e., if a species is not present in a certain year, it can't interact (Petanidou et al. 2008; Dupont et al. 2009; Trøjelsgaard et al. 2015), they have not incorporated into their analyses the fact that changes in presence/absence are one side of a spectrum of species abundance fluctuations per se. Given evidence that species abundances may drive a large part of network patterns, such that each species pair interacts in proportion to the product of their relative abundances (Vazquez et al. 2007; Vazquez, Chacoff & Cagnolo 2009), it logically follows that changes in the frequency of pairwise interactions across years that appears to be rewiring could, at least in part, be a response to changing abundances of either partner. Given that population sizes of wild pollinators are highly variable across time (Williams, Minckley & Silveira 2001) and that plant population abundance or floral density may also fluctuate due to environmental factors (Alarcon, Waser & Ollerton 2008), it is biologically likely that changes in abundance could in fact drive much of the observed rewiring. Thus the full consideration of the role of chance in driving apparent rewiring requires the consideration of the abundance of each species and how that changes across years.
In this study, we use a combination of a four-year field experiment and a null model to separate the roles of chance and behavioral plasticity in driving changes in the identity of interaction partners over time, or 'rewiring' as it has been referred to in the literature. We define chance as any changes in plant-pollinator interactions across years driven by abundance-based changes in random interaction patterns between plants and pollinators. We define behavioral plasticity as yearly changes in pollinators' preference for plants that is not driven by changes in plant or pollinator abundance across years. Our experimental network established a perfectly even species abundance distribution for the plant species, thereby allowing us to directly measure bees' preference for plant species by removing variation in partner choice that could be driven by variation in the plant species abundance distribution. We answer the following questions: (1) Given each bee species' level of preference for plant species, how much would bee species be expected to change plant species partners across years due to chance alone? To answer this we used a null model that maintained empirical annual abundances of bee species, and the overall (across all four years) preference of bee species for particular plants. (2) How much do the observed changes in preference over time compare with this null model? Any differential between the null and the observed reflects changes in preferences that are due to behavioral plasticity and not merely due to chance.

Materials and Methods

The four-year field experiment

In the fall of 2009, we established an experimental array of 17 native, perennial plant species located in a former old field near Cape May, New Jersey, USA (39.07 N, -74.46

W). Plants were established in 1 m^2 , single-species plots, each of which contained nine mature individual plants. We used a randomized block design with six replicate plots per species for a total of 102 plots. We used plant species that we expected to be attractive to native pollinators, based on our previous field studies in our study region, but we did not know the relative attractiveness of the plant species prior to establishing the experiment. We maintained the plots throughout the four-year experiment by weeding, watering, and replacing plants that died over the winter. Thus, given that the bee species in our study forage over 100 to 10,000 meters (Greenleaf et al. 2007) and our entire experimental array was 18 x 57 meters, our design creates a choice experiment for foraging bees. As plant species established over years, some filled out their plots differently, such that the original number of individuals per plot changed; however the total area covered by each plant species was standardized in all four years, such that the minimum was always 9 plants and the maximum area covered by those plants never exceeded the 1 m^2 area. We considered variation between these minimum and maximum areas to be one factor that may vary across plant species to influence pollinators' choice of plant species.

From May to September in each of 2010-2013, we netted all flower-visiting bees to each of the 102 plots on three separate days during the peak bloom of that plot. In order to capture bee species that forage at different times of day, plots were netted twice on each day, for 10 minutes in the morning and ten minutes in the afternoon. Within a single day, the order of plot sampling was randomized. We limited data collection to times when weather was sunny or partly cloudy, temperatures $\geq 16^{\circ}$ C, and mean wind speed was ≤ 3 m/s, with rare exceptions (less than 1% of sampling events occurred at greater wind speeds). All specimens were fully curated, barcoded, and identified to

species level by two professional taxonomists (J.A. and Jason Gibbs, Michigan State University, USA).

Analysis methods

(1) The null model: Given each bee species' level of preference for plant species, how much would bee species be expected to change plant species partners across years due to chance alone?

To construct the null model, we started with year by plant (Y x P) matrices for each bee species, with plant species in rows and years in columns, and cell values that represent the number of individuals of that species that were collected from each of the 17 plant species in our field experiment in each year. From this empirical matrix, we generated null matrices with two conditions. First, yearly abundances of the given bee species were maintained (i.e., the column sums of the null matrices matched the column sums of the empirical matrix), which prevents our null model from confounding changes in bee abundance with changes in bee foraging behavior. Second, the overall level of preference, where 'overall' means across all four years, that each bee species displayed for particular plant species is maintained, such that the row sums of the null matrices match the row sums of the empirical matrix. With this second restriction, we acknowledge that the extent to which a bee species has preferences at all, which is apparent from the evenness of row sum values in the empirical matrix, may influence our ability to detect rewiring that deviates from random. We also acknowledge that the identity of the plant species that it prefers is important to maintain in the null model. More specifically, a certain degree of preference is a necessary, but not sufficient,

condition for detecting non-random variation in preference. Thus our null model is not designed to measure preference itself, but instead to measure variation in preference over time that would be due to chance alone. For each bee species, the null model was iterated 1000 times to generate an expected value for the number of individuals of that species collected from each plant species. We then calculated, for each bee species, the mean Bray-Curtis dissimilarity in preference for plant species across all possible pairwise comparisons of years for that bee species. Thus because each bee species is a replicate in our analysis, our sample size for the number of comparisons varied somewhat across species, depending on how many years the species was present in the data set (1, 3, or 6)comparisons for species present in 2, 3 or 4 years, respectively). We calculated the SD for each bee species' across-years variation as a two-tailed, bootstrapped 95% confidence interval around the mean temporal dissimilarity for a given bee species (where the mean is calculated from 1,3, or 6 possible pairwise comparisons of years). 29 bee species were present in only one year, and these species were not analyzed because yearly changes in preference could not be determined. Six species were present in more than one year, but the structure of the Y x P matrix for these species was such that the mean dissimilarity value for pairwise comparisons of years was the same for all runs of the null model, indicating that our null model was not appropriate for these few, low-abundance species.

(2) How much do the observed changes in preference over time compare with this null model?

To answer this, we first calculated the empirical Bray-Curtis dissimilarity across years for each of the 48 bee species exactly as described above for the null model, but now using the observed (empirical) data matrix instead of a randomized null. Second, we compared our empirical dissimilarity results to the null expectation by calculating z-scores for each bee species (see below), and this allowed us to separate two hypotheses. The first hypothesis, which would be supported by empirical data that does not differ from the null, states that bees lack preference (i.e. they visit plants at random). The second, which would be supported if empirical data differs from the null, states that bee species show temporally variable preferences for plants that are not due to differences in plant abundance or yearly abundance fluctuations of the bee species. Thus our ultimate measure for each bee species is not that species' within-year preferences, but instead is the extent to which it changes its preferences across years compared to our null.

For each bee species we calculated a z-score as $z = (D_e - D_r)/SD_r$ where D_e is the mean empirical dissimilarity, D_r is the mean null dissimilarity, and SD_r is the standard deviation of the null distribution. This method produces 54 z-score values, one for each bee species, which were judged to be significant if greater than 1.96 or less than -1.96. Thus we define rewiring as a pattern by which bee species vary significantly more across years than is predicted by our null model. A z-score of >1.96 indicates that bee species show temporally variable preferences for plants. A z-score of <-1.96 indicates that bee species have preference for particular plants, but this preference does not vary across years, or that bee species consistently lack preference across years. A special case, in which a bee species has extreme preference for one plant species and has few or zero visits to others, could qualitatively be called fidelity but is not detected as such by our null model because the row sum restriction would cause the null to very closely mimic the empirical data. Both because we cannot separate two hypotheses for z-scores <-1.96, and because of the special case, we do not recommend the use of this method for detecting 'fidelity'.

Finally, we visualized the extent to which rewiring occurs more or less frequently among common or rare species by plotting the distribution of z-score values for all bee species along a continuum of species relative abundance.

Results

In our four-year field experiment, consisting of 2,448 collection events totaling 409 hours, we collected 7097 bees of 83 species. We conducted our analysis on the 48 bee species that we collected in more than one year, enabling us to calculate their change over at least one time step.

(1) Given each bee species' level of preference for plant species, how much would bee species be expected to change plant species partners across years due to chance alone? Our null model predicted some degree of annual dissimilarity for all species. However, our null model predicted a general trend of rare bee species varying more across years than common bee species (Figure 1). As expected, the null model also produced larger 95% confidence intervals for rare species, indicating that it is more difficult to differentiate empirical patterns from random chance when little data is available.

(2) How much do the observed changes in preference over time compare with this null model?

Unsurprisingly, all 48 bee species' choice of plant partners varied somewhat across years. However, for 19 bee species, this variation was not significantly different from what would be expected by chance. Another 29 species rewired, meaning that they varied more across years than what would be expected by chance.

However, we observed different patterns for rare and common bee species. When the empirical data was compared to the null, rare species either did not differ from the null, or in a few cases varied slightly more across years than expected, while almost all of the common species varied more than the null. However, compared with common species, we are less certain that the rare species rewired, because their z-scores were only marginally different from the null (Figure 2). This suggests that while rare bee species show a high degree of variation in plant species partners across years, we cannot say that they are rewiring, because in most cases they do not significantly deviate from the null. In contrast, annual variation among the more common bee species is evidence that those common bees rewire, because their deviation from the null is driven by higher than expected plasticity in plant species preferences.

Discussion

The few studies that have described temporal variation in pollination networks have found that the occurrence of interactions between individual plant-pollinator species pairs is highly variable, such that species 'rewire' their interactions (Alarcon, Waser & Ollerton 2008; Olesen *et al.* 2008; Petanidou *et al.* 2008; Dupont *et al.* 2009; Burkle & Alarcon 2011; Trøjelsgaard *et al.* 2015). However, because the probability of individual pairwise interactions is highly dependent on the abundance of both partners, and this varies from year to year (Vazquez *et al.* 2007; Vazquez *et al.* 2009; Blüthgen 2010), it has been difficult to determine whether the observed temporal patterns emerge because of biological factors such as pollinator behavioral plasticity, or simply because of chance, such that changes in species abundances alone predict much of the observed rewiring. Here we used a field experiment to control variation in plant species abundance within and across years, and a null model that controls for pollinator abundance and plant attractiveness in detecting changes in plant-pollinator interactions. We found that several species, primarily the common bees, varied more across years than would be expected by chance, suggesting that those species are showing behavioral plasticity (i.e. rewiring). At the same time, the majority of rare species did not differ from the null, suggesting that annual variation in interactions between plant species and rare bees is mostly due to chance.

Our results agree with previous studies that find much variability in the frequency of individual plant-pollinator species pairs across years (Alarcon, Waser & Ollerton 2008; Olesen *et al.* 2008; Petanidou *et al.* 2008; Dupont *et al.* 2009; Fang & Huang 2012), However, the key contribution of our study is that we establish a null expectation for the amount of annual variation in bee species' plant partners that would occur by chance alone as a function of bee species' overall preference for particular plants and year-to-year abundance fluctuations, such that we are able to define the annual dissimilarity measured in our empirical network that is not simply random, but that instead is driven by variation in bee species' choice of plant species across years. Beyond controlling overall preference and abundance fluctuations, there are additional components of our null model approach that make it particularly robust. First, because our experimental design controlled year-to-year variation in plant relative abundance, we were able to focus on the influence of pollinator foraging behavior alone in rewiring. Second, any non-linearity in how pollinators forage as a function of plant abundance is also taken out insofar as we controlled the relative abundance (per-plot area and minimum number of individuals) of plant species. Thus even though a similar null model approach to rewiring could be taken with observational data in more typical, skewed-SAD system, such an approach would require making, and then testing, the assumption that the relationship between bees per unit area of plant species is linear. While it is possible to account for across-plant sampling effects using statistical methods (Dorado *et al.* 2010; Chacoff *et al.* 2012), there are still biases associated with that approach because bees may choose plants differently if they are not equally available.

Furthermore, we found that the extent to which behavioral plasticity was detected depended on species relative abundance. When the empirical data was compared to the null, rare species either did not differ from the null, or in a few cases varied slightly more across years than expected, while almost all of the common species varied more than the null. This is unsurprising in light of our null model prediction that rare bee species will be highly variable across years based on chance alone; given this prediction it is in many cases not statistically possible to detect any higher level of variation among rare bee species than that generated by chance. This result is also interesting in light of the fact that rare species are often by default considered specialists in plant-pollinator networks; our results show that even those rare species show some variability across years, despite its lack of statistical significance in most cases (Blüthgen *et al.* 2008; Dorado *et al.* 2010).

The rewiring that we detect among common bee species could be driven by three co-occurring factors attributed either to plant species-level changes in attractiveness or to bee behavior, and we incorporate these three factors into our study design. First, even though we controlled relative abundance of plant species certain plant species could produce more flowers across years due to plant age, site or weather conditions (Aizen & Vazquez 2006). Changing floral area is an important potential driver of changes in pollinator visitation to plants across years, as floral area per se is often shown to be a primary determining factor in pollinator visitation patterns (Potts et al. 2003). Second, even if the number of individual plants or flowers per species remained consistent across years, the amount of resources per flower could vary, in that plants may respond to physical conditions by producing greater or lesser quantities or qualities of nectar or pollen (Aizen & Vazquez 2006). Thus our definition of rewiring is flexible in that it treats these factors as 'black-box attractiveness' factors that may vary within or across plants and across years and thus influence bee preference and behavior. Third, rewiring could also be attributed to bee species changes in behavior across years, either because of a change in the identity of the preferred plants, because of a change in the degree to which a bee species has preferences, or both. While our post-hoc exploratory analyses suggest that rewiring primarily occurs as a change in the identity of preferred plants, our model is not designed to distinguish between those possibilities. A final factor, which we did not incorporate into our study design, is that it is also possible that bees may alter their behavior across years depending on factors such as the presence of predators (Dukas & Morse 2003) or competitors for resources (Goulson, Hawson & Stout 1998). However, a more likely explanation for the level of change in bee species behavior that is detectable as rewiring is that attractiveness factors that vary across plant species and years are 'tracked' by bees that themselves may also change their preferences, or non-linear responses to those factors, across years (Pyke, Pulliam & Charnov 1977; Pleasants 1981; Potts et al. 2003; Roulston & Goodell 2011).

It is important to establish that rejecting the null hypothesis (i.e. species do not have preferences that change across years) is much more difficult for rare species than common species. Therefore, although we found that the majority of species that were rare did not rewire, this is primarily a statistical finding in that those species were so rare that detecting a significant result was not possible. This is a statistical issue that precludes any inferences about those bee species' true diet breadth (Dorado et al. 2010, Bluthgen et al. 2008).

A second point that is more biological than statistical involves different definitions of specialization in pollination ecology compared with network analyses, and what that means for the way our model detects rewiring. In pollination ecology (but not in network analysis), bee species dietary specialization is defined as female bees' restriction to collecting and provisioning larvae with pollen from only one or a few plant taxa (i.e., oligolecty, (Williams et al. 2010; Roulston & Goodell 2011). Network specialization, on the other hand, concerns only the pattern in which pollinator species visit plant species within a network—without distinguishing between different energetic sources. Thus a 'specialist' in a plant-pollinator network may be a male or female pollinator (bee or otherwise) that is found visiting very few plant species for either pollen or nectar. Likewise, an oligolectic (dietary specialist) bee may not be a network specialist, either because that species' specialization on the level of plant family (not on the level of plant species) is not apparent from the species-level interaction data in networks, or because data for both pollen and nectar visits are combined in the network. We found that two out of the three oligolectic species in our study showed behavioral plasticity, even though they are dietary specialists, and this is because they specialize on

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the level of plant family, but rewired across years to other plant species in the same family. Either way that specialization is defined, though, it is typically conflated with, and difficult to statistically separate from rarity (Dorado *et al.* 2010), and it does not preclude a species from rewiring in our model.

Previous studies of network variation across year have used statistical approaches to compare the fit of empirical plant-pollinator matrices across years (Alarcon, Waser & Ollerton 2008; Burkle & Irwin 2009) or to compare community similarity across space (Trøjelsgaard et al. 2015), finding much interannual variability in plant-pollinator interactions. A large majority of variation in the identity and frequency of interactions across years found by Burkle and Irwin (Burkle & Irwin 2009) was due to changing pollinator and floral abundances, although that study identified pollinators at the family level, so it is unclear exactly what species-level responses would have occurred and how those patterns would compare to those expected under null conditions. Similarly, Alarcon et al. (Alarcon, Waser & Ollerton 2008) found that the identity and frequency of plantpollinator interactions were similar in two of the three studied years, but in one year, variation in climatic factors influenced floral production. Because pollinators showed behavioral responses to changing floral production, this likely impacted the structure of the network. A third study compared community similarity across space, finding that the majority of differences between networks was due to changes in species composition (turnover effects) rather than to rewiring (Trøjelsgaard *et al.* 2015). While comparing networks separated by space is arguably less parallel to comparing temporal dynamics of a single network, it is still interesting to note that much variability across networks is driven by changing species abundances.

Our study presents a significant contribution to this literature in two ways. First, although other rewiring studies account for differential effects of species turnover, we acknowledge that species turnover is at the end of a spectrum of changes in abundance. We account for these changes in abundance with a null model that uses experimental data on bees' preference to account for changes in preference that deviate from random. Second, because we have a multiple year dataset that includes species-level identification of the main important pollinators, the bees, and these species comprise a wide range of relative abundances, we identify the extent to which relative abundance is related to interannual variation. We show that for less abundant species, rewiring is practically indistinguishable from random, but that for common species, behavioral plasticity can be statistically separated from changes in abundance, and this is evidence that common species rewire.

Figure 1. Comparison of year x plant matrices for empirical data (left) and the null model (right). We created these matrices for each of 48 bee species that were present in multiple years of the experiment. Each column in this year x plant matrix represents one of the four years of the field experiment; each row represents a plant species. The shading of the cells represent the frequency of visits by that bee species across plant species across years. The null model maintains the overall (across four years) abundance of a single bee species on each single plant species, and maintains the abundance of that bee species in each year. Given those restrictions, interactions between each single bee species and each of the 17 plant species are shuffled randomly across plants.



Figure 2. Expected Bray-Curtis dissimilarity variation across years according to species' relative abundances. Points represent the mean, and error bars the 95% confidence interval, of a null model that examined temporal dissimilarity in bee species' preferences for plants. The null model was implemented for each plant species individually (i.e. each point represents one bee species), and controlled bee species' across-years preferences for plants and yearly abundances of bee species. Expected dissimilarity tends to decline with abundance, indicating that simply examining dissimilarity without considering the null model would lead to biased conclusions. Confidence intervals decrease in size with abundance, resulting in greater power to detect non-random changes in preference across years.



Figure 2. Bee species abundance is positively correlated with non-random changes in preference across years. Along the x-axis, bee species are ranked in increasing order of overall (across-year) log abundance. The y-axis is the deviation of bee species from null dissimilarity (z-score). The dotted line represents the average dissimilarity of bee species in our null model, and the dashed lines above or below are located at -1.96 and 1.96, bordering an area that represents the two-tailed 95% confidence interval for z-scores. The steep increase in z-scores for abundant species is due to both greater differences in empirical and null model mean dissimilarities, and declines in the standard deviation across null model runs.



IV. References

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