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Species Abundance, Not Diet Breadth, Drives the Persistence of the Most Linked Pollinators as Plant-Pollinator Networks Disassemble

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ABSTRACT: Theoretical and simulation studies predict that the order of species loss from mutualist networks with respect to how linked species are to other species within the network will determine the rate at which networks collapse. However, the empirical order of species loss with respect to linkage has rarely been investigated. Furthermore, a species' linkage is a composite of its diet breadth and its abundance, yet the relative importance of these two factors in determining species loss order is poorly known. Here we explore the order of pollinator species loss in two contrasting study systems undergoing land-use intensification, using >20,000 pollinator specimens. We found that a pollinator species' linkage, as measured independently within plant-pollinator networks, positively predicted its persistence at human-disturbed sites in three of four analyses. The strongest predictor of persistence in all analyses was pollinator species abundance. In contrast, diet breadth poorly predicted persistence. Overall, our results suggest that community disassembly order buffers plant-pollinator networks against environmental change by retaining the highly linked species that make a disproportionate contribution to network robustness. Furthermore, these highly linked species likely persist because they are also the most common species, not because they are dietary generalists.

Keywords: biodiversity, ecosystem stability, mutualist network, mutualistic web, plant-pollinator interaction.

Introduction

Ecological communities can be characterized as networks of species (nodes) connected through interactions (links). It is well known from theoretical and modeling studies that networks of mutualists are sensitive to the loss of species that are linked to a large number of other species (Sole and Montoya 2001; Dunne et al. 2002; Fortuna and Bascompte 2006). Specifically, simulations show that when the species that are highly linked are lost first, secondary extinctions of other species accrue rapidly. Conversely, when highly linked species are lost last, secondary extinctions are minimized, making the network relatively robust (Memmott et al. 2004; Verdu and Valiente-Banuet 2008; Kaiser-Bunbury et al. 2010). Such predictions have profound implications for the stability of real communities facing anthropogenic disturbance because the functional impact to the entire system will depend on which species are lost first and how their loss propagates to other species. Despite these clear theoretical predictions, however, the actual order in which mutualist species are lost from real networks is poorly known (Bascompte and Stouffer 2009; Wardle et al. 2011).

A species' linkage, as it is observed within mutualist networks, is actually a composite of two factors. Observed linkage is measured as the number of partner species observed interacting with a focal species—for example, the number of plant species that a pollinator species is observed to visit. Thus, observed linkage increases with the pollinator's diet breadth. But observed linkage also reflects the underlying abundance distributions of the species involved: if researchers encounter more individuals of an abundant species, then they will likely encounter it interacting with more partner species by chance alone. In the mutualist network literature, this bias has often been ignored, thereby confounding diet breadth and abundance (Vázquez et al. 2009; Blüthgen 2010). The roles of abundance and diet breadth can be separated by statistically correcting the observed linkage for each species by the number of individuals recorded for that species, thereby obtaining an abundance-corrected measure of diet breadth (Vázquez 2002; Kleijn and Raemakers 2008; Chacoff et al. 2012).

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The effect of land-use change on mutualist networks has been previously investigated from several perspectives. Some studies have explored the role of diet breadth in predicting mutualist response to disturbance but without assessing observed linkage or species loss order, thus leaving the implications for network disassembly unstudied (Vázquez 2002; Williams et al. 2010). Other studies have examined how entire networks and network metrics such as connectivity and nestedness change across disturbance gradients but without focusing on the loss order of species themselves (Passmore et al. 2012). Yet another line of work has focused on the persistence of interactions rather than on the persistence of species. For example, both Aizen et al. (2012) and Burkle and Knight (2012) found that interaction networks within progressively smaller habitat fragments are nested, suggesting that the persistence of interactions increases with the mean linkage of the two partners. This result was originally predicted by Ashworth et al. (2004) on the grounds that species with fewer links will be more vulnerable to habitat loss and, thus, so too will be the interactions in which they participate. These same two studies found that dietary generalists persist better as habitat is lost, but they did not correct diet breadth for sampling effects; thus, the results could simply reflect the greater persistence of abundant species (Aizen et al. 2012; Burkle and Knight 2012). In addition, the measures of linkage and diet breadth came from the same sites where species loss order was assessed, introducing some circularity into the analyses. Finally, in one study conducted over a century of land-use change, pollinators with smaller diet breadth were found to go locally extinct more often; however, pollinators were collected only from a subset of all available plants, making it difficult to separate extinction from changes in foraging preferences (Burkle et al. 2013).

This article represents an advance over previous work in two important ways. First, we account for statistical sampling biases in our measure of diet breadth and thus can more fully separate the relative importance of abundance and diet breadth in driving any effect that is observed for linkage. Second, we measure the network traits (linkage, abundance, and diet breadth) at sites independent of those where we measured species persistence, thereby avoiding circularity in our analysis. Specifically, we ask two questions: (1) Are the pollinator species that persist at disturbed sites more or less highly linked within the plant-pollinator network? The empirical answer to this question is needed to interpret theoretical predictions about the robustness of entire plant-pollinator networks to land-use change. (2) Are the pollinator species that persist at disturbed sites more or less abundant within the plant-pollinator network and likewise have greater or lesser diet breadth? The answer to this question clarifies the biological mechanism behind any effect found for linkage. We expected that highly linked species should persist better than average, because linkage is a composite of abundance and diet breadth, both of which should increase persistence. We further expected that abundance would have stronger positive effects than diet breadth on persistence, since common species generally persist better than rare species as human disturbance increases and most previous studies concluding that dietary generalists persist better have not corrected for sampling effects.

Methods

Study Systems and Study Design

We used parallel study designs in two contrasting ecosystems, one in New Jersey and one in California. In each study system, we used a two-part study design, first measuring species traits (observed linkage, abundance, and diet breadth) within relatively intact plant-pollinator networks and then recording species loss order independently at a separate set of sites arrayed along a gradient of increasing human land use. In each study system, we defined a reference habitat type known to support a diverse community of native pollinators. In New Jersey, we used the spring ephemeral plant community found in the native deciduous forest as our reference habitat type. This habitat supports a diverse and characteristic native pollinator community (Motten 1986) but in our study system is becoming fragmented and replaced by agriculture and suburban/urban development. In California, we used a mosaic of small-scale diversified organic farms and seminatural habitats (native chaparral, oak savannah, mixed riparian vegetation, and seminatural grasslands) as the reference habitat type. This habitat mosaic supports a high diversity of native bees but is being replaced by high-intensity monoculture agriculture, which supports few native bee species (Kremen et al. 2002).

We measured pollinator species traits (observed linkage, abundance, and diet breadth) by sampling plant-pollinator networks within the most extensive patches of our reference habitat type available within each study area (hereafter called reference sites). Because our goal was to measure species-level traits, which requires averaging across context-dependent variation in pollinator population sizes and foraging choices, we used multiple reference sites covering a large total area (3 in New Jersey totaling 23 ha, 13 in California totaling 26 ha). We independently measured pollinator species loss order at a separate set of sites, which consisted locally of the reference habitat type but were arrayed along steep gradients of land-use intensity (hereafter called gradient sites; table 1). Thus, we use a space-for-time substitution in exploring the ecological effects of

Table 1: Characteristics of the gradient sites where pollinator community composition was measured

Study system	No. sites	Local habitat type	Canopy closure (%)	Spring ephemerals, as % of total floral abundance	(Semi)natural land cover types surrounding site	Proportion (%) of (semi)natural land cover at 500-m radius
New Jersey	13	Mature deciduous forest	90 ± 2 SD	89 ± 16 SD	Deciduous forest	6–99
California	17	Field border of diversified organic farm	NA	NA	Native chaparral, oak savannah, mixed riparian vegetation, and seminatural grasslands	0–90

Note: NA = not applicable. SD = standard deviation.

land-use change, as done by most similar studies (Winfree et al. 2011). All gradient sites were at least 3 km apart, which is beyond the typical flight ranges of all but the largest pollinator species in each study system (Greenleaf et al. 2007).

Data Collection

Timing of data collection. Within the eastern deciduous forest (New Jersey), peak plant-pollinator activity occurs in April and May when the spring ephemeral wildflowers bloom. Once the canopy closes, forests support few flowers and few pollinators (fig. A1; figs. A1–A4 are available in the online appendix); thus, we collected all of our data between April 10 and June 1, when the canopy closed (table 1). In the California ecosystem, pollinators reach their peak abundance during the summer; thus, we collected all of our data between June 4 and August 8.

Reference sites. The purpose of data collection at reference sites was to develop quantitative network data on which pollinator species visit which plants and in what abundances. To accomplish this, a researcher walked throughout the site inspecting flowers of all plant species in bloom and using a hand net to collect all insects that were visiting flowers (subject to the taxonomic criteria described below). The plants from which insects were collected were identified to the species level in the field; if that was not possible, then a herbarium specimen was collected to identify in the laboratory. Reference site network data were collected by hand net for a total of 201 person-hours in New Jersey and a total of 104 person-hours in California. Additional data on pollinator abundance at reference sites were collected in California using a passive sampling method, pan traps (Westphal et al. 2008), in order to match the gradient site data from California, which were also collected by pan trap (see below). On each of four sampling dates per reference site in California, 30 pan traps (178-mL plastic bowls; Solo, Kernersville, NC) were placed in triplets (one each of blue, yellow, and white) at 10-m intervals along a transect of 90 m through the center of the site. Traps were placed at the site before 08:00 and removed at 15:00–15:30.

Gradient sites. At gradient sites, where our purpose was to assess the presence and abundance of pollinator species, we collected pollinating insects using standard pan-trapping and hand-netting methods (Westphal et al. 2008). In New Jersey, we established one 0.5-ha plot at each of the 13 gradient sites. We collected data within these plots on four different days between April 10 and June 1, 2006, with site visits organized into rounds of 9–13 days each, such that all sites were visited in each round prior to beginning the next round. Data were collected only on days suitable for pollinator activity, defined as sunny to partly cloudy, with temperatures no less than 14°C (first round; later rounds used temperatures of at least 15° and 16°C as ambient temperatures increased) and wind speeds of less than 2 m/s. At each site visit, flower-visiting insects were hand-netted for 60 min, and 39 pan traps (178-mL plastic bowls; Solo) were placed in a 10-m grid throughout the plot, alternating the colors fluorescent blue, fluorescent white, and fluorescent yellow. Traps were left in place for either 2 h (rounds 1, 2) or 4 h (rounds 3, 4). In California, we established two 50 × 1-m data-collection transects at each site, placing these along two perpendicular edges of organic crop fields. Within each transect, we placed 18 pan traps (355-mL plastic bowls; Solo), six each of blue, yellow, and white. All pans were set out on the morning of the same date (June 22, 1999) by three field teams operating simultaneously, and pans were collected in the same site order approximately 24 h later.

Filtering of pollinator species to be used in the analysis. In both study systems, we collected insects visiting flowers; hence, these insects are technically flower visitors rather than pollinators. We hereafter use the term "pollinators," however, because our analyses are based on the taxa known to be the primary pollinators in each system. In California, we collected only bees (Hymenoptera: Apiformes), which make up the dominant pollinator taxon in that system (Kremen et al. 2011). In New Jersey, in addition to bees, we collected butterflies (Lepidoptera) and flies (Diptera) because these are important pollinating taxa in spring

woodlands (Motten 1986). A taxonomic filtering step was required in New Jersey in order to extract from the pan traps only the fly species known to visit flowers, since unlike bees and butterflies, only a minority of flies visit flowers. We used the flies we collected by hand net, all of which were visiting flowers, to identify two families (Bombyliidae and Syrphidae) plus two genera within a third family (Tachinidae: *Epalpus* and *Gonia*), which collectively accounted for 96% of the fly specimens we collected from flowers. We then extracted only these taxa of flies from our pan-trap collections.

A second filtering step related to habitat affinity was necessary for New Jersey only. Many pollinator species are not positively associated with the native forest habitat type but instead are more abundant in human-disturbed habitats. This is true in forested biomes generally (Winfree et al. 2011), in our previous work in this region (Winfree and Kremen 2009), and in the present study (fig. A2). The presence of such disturbance-associated species would hinder our ability to detect species loss across a forest fragmentation gradient. Thus, we did a preliminary analysis with our New Jersey data to identify the forest-associated species to use in our analyses. This step was not necessary in our California system, where neither our previous work nor data from the present study found any species that increase along the human disturbance gradient (Winfree and Kremen 2009; Klein et al. 2012; fig. A2).

To identify the species that are associated with forest habitat in New Jersey, we used a nonmetric multidimensional scaling (NMDS) ordination of pollinator species collected at the three reference sites, as compared with the species collected at three sites situated in highly disturbed, urban, and agricultural habitats. Data were collected at the disturbed sites using the same methods and over the same time period as the gradient site data collection. The disturbed sites are not part of the gradient site analysis, thus making the NMDS ordination independent of that analysis. In total, the data used in the NMDS ordination consisted of 2,197 specimens of 159 species. Ordination analyses were performed using the vegan and MASS packages in R (ver. 2.13; R Core Team 2012). We used the NMDS to ordinate sites by their pollinator community composition and then used the pollinator species scores to determine which species were associated with the forested (reference) sites. A one-dimensional solution was selected, because (1) runs using higher dimensionality showed that the forested and disturbed sites separated along only one axis, (2) species scores generated by the NMDS were more stable across runs for the one-dimensional solution compared to the two-dimensional solution, and (3) the final stress of the one-dimensional solution was acceptable at 0.11. In this solution, forested sites had scores no less than 0.149, and disturbed sites had scores no greater than −0.102. Therefore, we defined the pollinator species with NMDS scores no less than 0.149 as forest associated. Several species with scores slightly less than 0.149 are known a priori to be common in human-disturbed areas (e.g., *Bombus impatiens* and *Xylocopa virginica*), suggesting that our threshold was biologically reasonable.

Finally, in both study systems, we excluded the species for which we collected less than three records of individuals visiting plants at reference sites, on the grounds that the linkage for such infrequently collected species could not be analyzed in an unbiased way. This resulted in retaining 45 out of 91 species in New Jersey and 25 out of 59 species in California.

Analytical Methods

All of our analyses were designed to determine whether the pollinator species persisting at gradient sites are different from random with respect to the three species traits observed linkage, abundance, and abundance-corrected linkage, which was our measure of diet breadth. We calculated values for the species traits based on the data we collected from the relatively intact plant-pollinator networks at reference sites. Observed linkage was defined as the number of plant species from which a given pollinator species was collected, while abundance was defined as the number of individuals collected for the pollinator species. Diet breadth was estimated as abundance-corrected linkage using the Chao2 estimator (Chao et al. 2009); here abundance is the number of individual pollinators collected from flowers and used to estimate diet breadth. Chao2 is a nonparametric asymptotic estimator that, in this usage, approximates the number of plant species from which a given pollinator species would be collected, given sufficient sampling. Thus, it compensates, at least in part, for the inherent bias in network data that can cause poorly sampled species to appear more specialized in their diet than they may truly be. Chao2 performs well against other asymptotic estimators in comparative tests of accuracy, particularly at small sample sizes (Walther 2005). The three species traits are portrayed in figure 1.

We used both a permutation model approach and a traditional statistical test to explore the question of nonrandom species loss with regard to linkage, abundance, and diet breadth. First, we used a permutation model to ask whether the pollinator community composition found at gradient sites has different properties than would be expected with random persistence. This approach has the advantage of including information about species abundance at gradient sites; however, because the model permutes species identities across sites and the three traits go along with identity, it cannot fully separate the relative effects of the three traits on species persistence. Second,

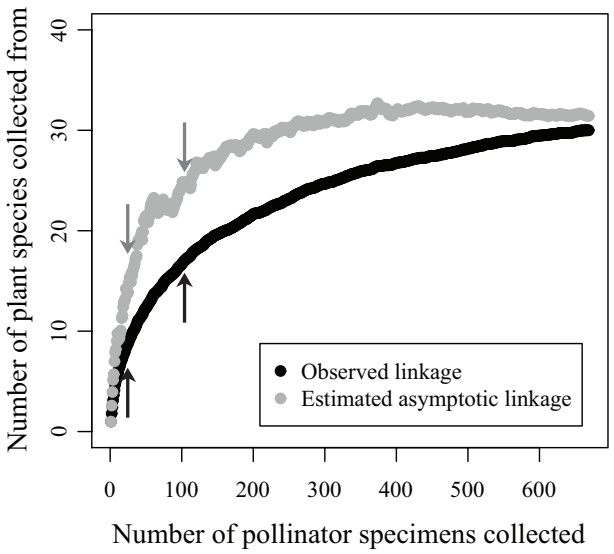

Figure 1: Relationship between the number of pollinator specimens collected and the values obtained for observed linkage and asymptotic linkage (diet breadth) as estimated by Chao2. The data shown are for *Halictus tripartitus* in the California plant-pollinator network. This is a particularly well-sampled species, thus the observed and asymptotic linkages converge at the far right. For less well-sampled species, values will be less similar and thus the Chao2 correction more necessary. Arrows indicate our sample sizes (mean number of specimens per species) for New Jersey (25) and California (104).

we used a generalized linear mixed model (GLMM) to ask whether each of the species traits predicted the persistence of pollinator species at gradient sites. This model used a binary response (species presence/absence at gradient sites) and thus could not incorporate information about abundance. However, it has the advantage of being better able to separate the relative effects of the three predictors.

Community-Level Permutation Model

The basic approach of the permutation model is to test whether the pollinator communities we observed at the gradient sites were different from randomly assembled communities of the same species richness with respect to the mean observed linkage, abundance, and diet breadth taken across the individual pollinators in the community. First, null communities of the same species richness as the actual gradient sites were created by randomly permuting the complete list of species found across all gradient sites. Because the species traits (observed linkage, abundance, and diet breadth) are attributes of the species, they change along with species identity, thus creating null communities for which the species traits are randomized. For each of the null communities thus created, we calculated one value for each species trait as the abundance-weighted mean across the pollinator species present (hereafter referred to as mean trait). Significance tests compared the mean trait calculated for the empirical community to the distribution of mean traits for the null communities with the same species richness. All permutation model analyses were conducted in R, version 2.13, and statistical tests were based on 2,000 permutations.

Community Persistence at the Gradient Sites as a Whole. We first asked whether the pollinator communities found at the entire set of gradient sites had higher or lower linkage, abundance, or diet breadth as compared to null communities. This could occur if species with a given trait were more frequent at gradient sites, more abundant, or both.

Community Change across Richness and Land Cover Gradients. Second, we asked whether the pollinator communities changed in a directional way across the gradient itself. Such an effect could occur if species with a given trait were the first to be lost across the gradient, became less abundant across the gradient, or both. We explored pollinator community changes across gradient sites in terms of both species richness (i.e., what happens as species are lost from the community; this matches the approach taken by the theoretical literature) and land cover (i.e., what happens as natural and seminatural habitat is lost). These questions are not redundant, given the modest correlation between land cover and pollinator species richness ($R^2 = 0.45$, $P = .01$ in New Jersey; $R^2 = 0.17$, $P = .10$ in California).

To conduct the land cover analysis, we used ArcGIS 9.0 (Environmental Systems Research Institute, Redlands, CA) to measure the proportion of natural or seminatural habitat (table 1) surrounding each gradient site. We determined the spatial scale at which surrounding land cover best explained changes in pollinator species richness by regressing richness against land cover measured at six different radii, ranging from 200 to 1,700 m (Holland et al. 2004). In both study systems, the highest R^2 value was found at a 500-m radius around the study plots. We therefore used land cover measured at this scale in all further analyses.

Species-Level GLMM Analysis

We used a GLMM with a binomial response and logit link (R package lme4, function glmer) to predict species presence/absence at gradient sites as a function of the three species traits (observed linkage, abundance, and diet breadth). In order to make the results comparable across predictors and interactions, all variables were standardized prior to analysis by subtracting the mean and dividing by the standard deviation. Because observed linkage is a com-

posite of the other two traits, we did not put all three traits in the same model. Rather, we first ran an observed linkage model that included as fixed factors the observed linkage for each pollinator species, species richness (or land cover) at each gradient site, and the richness (or land cover) by linkage interaction. Species was a random factor. The response variable was the presence/absence of each species at each gradient site. The results of the observed linkage model can be interpreted with respect to the theoretical literature about network disassembly, which is based on observed linkage.

The second GLMM model included abundance and diet breadth, which allowed us to quantify which factor is driving the effects found for observed linkage model. The abundance and diet breadth model included the following fixed factors: abundance, diet breadth, species richness (or land cover), richness (or land cover) by abundance interaction, and richness (or land cover) by diet-breadth interaction. Species was a random factor. The response variable was the presence/absence of each species at each gradient site. This model can be interpreted in terms of which factor is driving the effects found for observed linkage.

Species Persistence at the Gradient Sites as a Whole. In the statistical models above, main effects for observed linkage, abundance, and diet breadth represent the predictive value of each species trait in terms of pollinator species frequency at the gradient sites as a whole.

Species Loss Order across Richness and Land Cover Gradients. In the statistical models above, the interaction terms between each species trait and either species richness or natural land cover at gradient sites represent the predictive value of the species trait in terms of the order of pollinator species loss across the gradient. Thus, richness (or land cover) per se is not a variable of interest, but the interaction term is. For example, a significant positive richness × linkage interaction would mean that the more linked pollinator species are likely to be found at the more species-rich sites, indicating that highly linked species are the first to be lost as richness declines.

Results

Our analyses were based on a total sample size of 20,570 pollinator specimens, broken down as follows. In New Jersey, our plant-pollinator network of forest-associated pollinator species from reference sites contained 45 pollinator species, 51 plant species, and 1,016 interaction records (specimens) representing 291 unique species-species pairwise interactions. In California, our plant-pollinator network from reference sites contained 25 pollinator species, 69 plant species, and 2,610 interaction records (specimens) representing 277 unique pairwise interactions. Another 11,055 specimens of these same species were collected by pan trap at reference sites and used to estimate species abundance. At gradient sites in New Jersey, we collected 32 pollinator species, comprising 1,604 specimens. At gradient sites in California, we collected 20 pollinator species, comprising 3,104 specimens (data are deposited in the Dryad Digital Data Repository: http://dx.doi:10.5061/dryad.qq67h [Winfree et al. 2014]).

An examination of the associations among the different species traits as measured at reference sites showed that for both systems the use of the asymptotic linkage estimator (Chao2) to assess diet breadth reduced the correlation between pollinator abundance and the number of plant species visited by that pollinator species. In New Jersey, the Pearson's correlation between observed linkage and abundance was $r = 0.43$, while the correlation between abundance and diet breadth (Chao2) was reduced to $r = 0.22$. In California, the correlation between observed linkage and abundance was $r = 0.74$, while the correlation between abundance and diet breadth was $r = 0.56$. Observed linkage and diet breadth were positively correlated in both systems (New Jersey, $r = 0.61$; California, $r = 0.91$).

Community-Level Permutation Model

Community Persistence at the Gradient Sites as a Whole. The pollinator species that were more highly linked within the complete plant-pollinator network persisted best at gradient sites in California but not in New Jersey (table 2; fig. 2*A*, 2*B*; for California but not New Jersey, the data points as a whole are above the null communities ± confidence interval [CI]). Pollinator species that were more abundant within the complete plant-pollinator network persisted best in both study systems (table 2; fig. 2*C*, 2*D*). Species with greater diet breadth persisted better in California but not in New Jersey (table 2; fig. 2*E*, 2*F*).

Community Change across Richness and Land Cover Gradients. There were no significant patterns of species loss order across the gradient sites in either New Jersey or California with respect to either declining species richness or decreasing land cover by natural and seminatural habitats (table 2). Although in some cases there were apparent trends across the gradient sites (fig. 2 for richness, fig. A3 for cover), in fact the empirical values for Pearson's *r* obtained for the correlation between species richness at a site and the mean pollinator trait value at that site do not differ from the distribution of Pearson's *r* values produced by the permutation model (fig. A4).

Table 2: Results of the permutation model

Study system, fixed factor	Persistence at gradient sites as a whole (effect size, *P*)[a]	Changes with richness across gradient (*r*, *P*)[b]	Changes with land cover across gradient (*r*, *P*)[b]
New Jersey:			
Linkage	+0.2, .49	.50, .09	.71, .16
Abundance	**+5.7, .005**	−.35, .26	−.52, .30
Diet breadth	+.1, .71	.43, .26	.63, .31
California:			
Linkage	**+4.3, .01**	−.57, .27	−.61, .34
Abundance	**+41.6, .009**	.44, .48	.50, .54
Diet breadth	**+2.8, .02**	−.58, .24	−.62, .28

Note: Significant effects are in bold.

[a] Effect sizes represent the factor by which the linkage, abundance, or diet breadth for the mean individual observed at gradient sites differed from the random null. *P* values represent the probability that the mean individual sampled at gradient sites would have a value for observed linkage, abundance, or diet breadth at least as extreme as that observed if species were distributed randomly across gradient sites.

[b] Pearson's *r* are reported for the correlation between the values for each species trait observed at the gradient sites and the species richness or land cover at the site.

Species-Level GLMM Analysis

Species Persistence at the Gradient Sites as a Whole. The GLMM linkage model showed that a pollinator species' linkage within the complete plant-pollinator network positively predicted its frequency at gradient sites in both study systems (table 3). Likewise, the GLMM abundance and diet breadth model showed that a pollinator species' abundance within the complete plant-pollinator network positively predicted its frequency at gradient sites in both study systems (table 3). In contrast, diet breadth was a negative, although nonsignificant, predictor in both study systems (table 3). The estimated effect of changing each of the three focal variables (observed linkage, abundance, and diet breadth) is portrayed graphically in figure 3. The effect of each predictor on species presence was visualized using the estimate and the 95% CI of the slope of the relationship, with other variables held at their means (on the log-odds scale). For biological interpretability, lines with these three slopes were drawn through the center point of the model (representing the mean of the predictor and the mean log-odds abundance, and thus, at this point, the CI are zero) and then transformed into probabilities. Thus, the 95% CIs show the range of possible slopes that the relationship could take. Where the CI curves have one positive slope and one negative slope (as in fig. 3*E*, 3*F*), we are not confident of the true sign of the relationship (i.e., the relationship is not statistically significant).

Species Loss Order across Richness and Land Cover Gradients. Neither observed linkage, abundance, nor diet breadth predicted species loss order as pollinator species richness declined across the gradient sites in either New Jersey or California (table 3). Neither abundance nor diet breadth predicted species loss order as land cover by natural and seminatural habitats declined across the gradient sites in either New Jersey or California. Observed linkage was positively associated with natural land cover in New Jersey but not in California (table 3). This means that in New Jersey, the more highly linked pollinator species were lost first as natural land cover declined.

Discussion

Network theory and modeling studies predict that the loss of highly linked species from mutualist networks leads to rapid network collapse, whereas persistence of these same species makes networks relatively robust in terms of secondary species loss (Dunne et al. 2002; Memmott et al. 2004; Verdu and Valiente-Banuet 2008; Kaiser-Bunbury et al. 2010). Here we show that the most linked pollinator species persist better than average at sites undergoing land-use intensification (gradient sites) in three of four analyses (tables 2, 3; figs. 2, 3). This result is broadly consistent with two previous studies that, while using different methods and analytical approaches, also found that the most linked pollinators persist best at sites undergoing land-use change (Aizen et al. 2012; Burkle and Knight 2012). In a second set of analyses, we explored the order of pollinator species loss across the gradient sites with respect to both species richness and the loss of (semi)natural land cover. In seven of eight such analyses, there were no significant patterns found, but in one analysis (changes with land cover at the species scale in New Jersey), the most linked pollinator species were lost first (tables 2, 3). Thus, overall, we found strong evidence that the more linked pollinator species persist well at the gradient sites as a whole but,

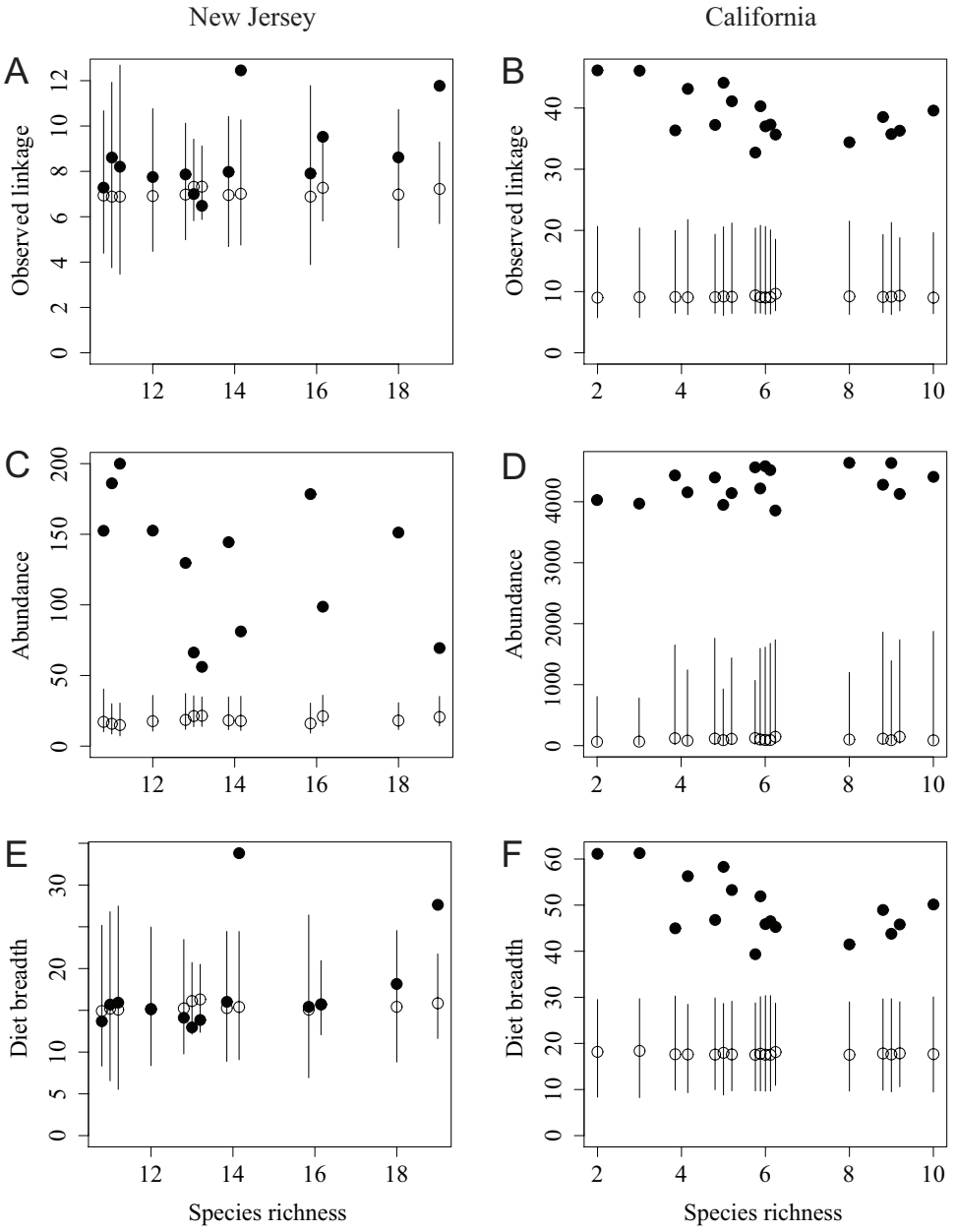

Figure 2: Results of the community-level permutation model. The abundance-weighted mean values for observed linkage (*A*, *B*), abundance (*C*, *D*), and diet breadth (*E*, *F*), as calculated across the pollinator species found at each gradient site in New Jersey (*left column*) and California (*right column*), are shown against species richness at the site. Filled circles are the data; open circles are null communities generated by the permutation, with 68% quantiles (analogous to 1 standard deviation). Circles for sites with the same species richness are jittered slightly for clarity.

nested within that result, weak contrasting evidence that in some cases the more linked species can be sensitive to natural habitat loss.

An important contribution of our study is that we made an effort to separate the roles of abundance and diet breadth in driving the persistence of the highly linked species. A species can be highly linked because it is abundant, has a wide diet breadth, or both. Abundance can have a strong influence on observed linkage because, by chance alone, more abundant species are likely to be observed interacting with more partners. While this point is not lost on ecologists who do network analysis, it has

Table 3: Results for the generalized linear mixed model (GLMM)

Study system, fixed factor	Persistence at gradient sites as a whole (estimate, *P*)[a]	Changes with richness across gradient (estimate, P)[b]	Changes with land cover across gradient (estimate, *P*)[b]
New Jersey:			
Linkage	**+.68, .01**	+.21, .12	**+.36, .006**
Abundance	**+1.87, <.001**	+.19, .58	+.19, .58
Diet breadth	−.44, .09	+.08, .56	+.08, .56
California:			
Linkage	**+1.22, .008**	−.36, .16	−.11, .66
Abundance	**+4.20, .002**	+1.12, .29	1.12, .29
Diet breadth	−.46, .22	−.39, .19	−.40, .19

Note: This table reports only the results relevant to our research question, in parallel to table 2. See table A1 (tables A1, A2 are available in the online appendix) for complete output from all GLMM models. All variables were standardized prior to analysis. Significant effects are in bold.

[a] The main effect for each species trait (using the model with richness as a covariate; results were similar with cover as the covariate; see table A1).

[b] The interactions between each species trait and richness or land cover; these interactions are analogous to the *r* values reported for the community-level permutation model in table 2.

nonetheless not been accounted for in most such analyses to date (Blüthgen et al. 2008; Kleijn and Raemakers 2008; Dormann et al. 2009; Vázquez et al. 2009; Blüthgen 2010; Dorado et al. 2011). Thus, our finding that the most linked species persist best could merely reflect the persistence of abundant species, which are expected to be at lower risk of extirpation (Davies et al. 2000; Verdu and Valiente-Banuet 2008). In addition, abundant species might be more readily detected at gradient sites, thus creating an additional bias toward finding that abundant species persist best. Alternatively, even after the effect of abundance is factored out, the species with greater diet breadth might persist best (Bond 1994; Steffan-Dewenter and Tscharntke 2000), as has been proposed on the grounds that such species are more readily able to find suitable resources in disturbed habitats (Vázquez 2002; Vázquez and Aizen 2003).

We found that abundance was the strongest predictor of pollinator persistence at gradient sites in all of our analyses and in both study systems (tables 2, 3; figs. 2, 3). We therefore conclude that a species' abundance not only predicts its persistence in human-disturbed areas but is likely the factor driving the persistence of the highly linked species as well. In contrast, results for diet breadth were largely nonsignificant, suggesting that it is less important in its own right and as a component of observed linkage (tables 2, 3; figs. 2, 3). Furthermore, in the GLMM model, which could separate the relative effects of abundance and diet breadth, the sign for diet breadth was negative (although nonsignificant; table 3). This result shows that once the positive effect of abundance is accounted for, species with greater diet breadth, if anything, persisted somewhat less well than more specialized species. Finally, in our second set of analyses, to explore patterns of species loss across the gradient sites themselves, we found nonsignificant results for both abundance and diet breadth, similar to the case for observed linkage (tables 2, 3). Overall, our findings contrast with previous studies that have concluded that mutualists with greater diet breadth persist better (Aizen et al. 2012; Burkle and Knight 2012). Perhaps this is because these previous studies did not correct their measures of diet breadth for sampling effects due to abundance.

Several unanswered questions remain as interesting areas for future study. First, as is the case for most studies of plant-pollinator networks, we were unable to measure actual pollination for all of the species pairs in our network and so use flower visitation as a proxy for actual pollination. Future work could explore whether the results reported here are also found for pollination networks. We expect this to be the case because, in general, flower visitation is a strong predictor of pollination in networks such as those we studied (Vázquez et al. 2005). Second, in our New Jersey study only, we applied a filter to the pollinator data prior to analysis, such that we explored species loss order for only the pollinator species determined a priori to be positively associated with the native vegetation cover (deciduous forest). If we had not applied this filter, then we would have found that many presumably disturbance-associated pollinator species were gained across the gradient of forest loss (fig. A2). It would be interesting to know how these disturbance-associated pollinators integrate into the complete plant-pollinator network. However, we were unable to investigate this further in this study because, by definition, disturbance-associated pollinators were not present at the extensive forested sites where we collected our plant-pollinator network data.

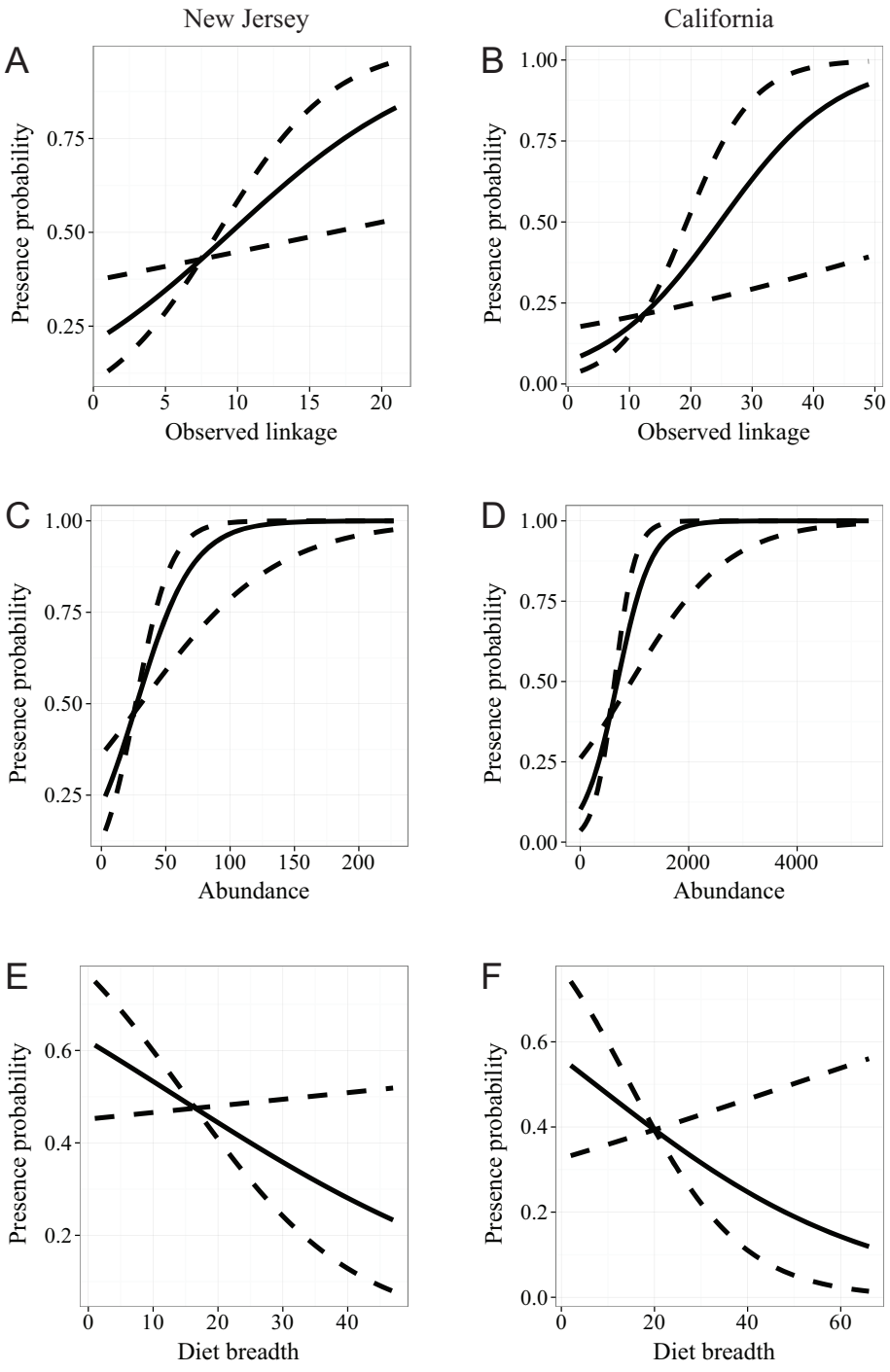

Figure 3: Species persistence at the gradient sites as a whole (generalized linear mixed model). Solid lines show the predicted effects of observed linkage (*A*, *B*), abundance (*C*, *D*), and diet breadth (*E*, *F*) on the probability pollinator that will be present at a gradient site. Effects of each predictor are calculated by holding the other variables constant at their mean values.

Finally, while we believe that we used the best available methods to remove the effect of abundance from observed linkage and thereby separate it from diet breadth, there are some subtleties that merit further work. Our ability to statistically separate the effects of observed linkage, abundance, and diet breadth on species persistence depends on the strength of the intercorrelations among these predictors as calculated across pollinator species. The use of an asymptotic estimator, Chao2, reduced the correlation between pollinator abundance and the number of plant species visited in both study systems (i.e., diet breadth as calculated by Chao2 was less correlated with abundance than was observed linkage). However, in the California system, the remaining correlation between abundance and diet breadth was still strong, and in both systems, observed linkage and diet breadth remained positively correlated. There are several possible reasons for these correlations. Most obviously, the sampling at reference sites will always contain some rare species for which we have incomplete knowledge of total plant host use. The diet breadth estimates for these species are not and cannot be completely corrected for sampling effects (see left-hand side of fig. 1). Thus, to some extent, we cannot avoid the accumulation of observed links with increased sampling effort, that is, abundance. However, there are two other reasons why observed linkage, abundance, and diet breadth might be positively correlated, and neither is a sampling effect. First, at high levels of sampling effort, observed linkage and diet breadth converge because they have both reached the asymptote of true diet breadth (see right-hand side of fig. 1). Second, and this is a biological rather than statistical reason, the dietary generalists might indeed be the most abundant species.

In conclusion, we show that as pollinator species are lost with intensifying land use, the species that are most linked within plant-pollinator networks persist well in most cases. The reason for their persistence is that they are the most abundant species. Thus, abundance, not diet breadth, appears to be the driving force in observed linkage as pollinator communities disassemble.

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