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LETTER

Dispersal enhances beta diversity in nectar microbes

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Abstract

Dispersal is considered a key driver of beta diversity, the variation in species composition among local communities, but empirical tests remain limited. We manipulated dispersal of nectar-inhabiting bacteria and yeasts via flower-visiting animals to examine how dispersal influenced microbial beta diversity among flowers. Contrary to the prevailing view that dispersal lowers beta diversity, we found beta diversity was highest when dispersal was least limited. Our analysis suggested that this unexpected pattern might have resulted from stronger priority effects under increased dispersal. Dispersal is highly stochastic, generating variability in species arrival history and consequently the potential for community divergence via priority effects, in these and likely many other microbial, plant, and animal communities. Yet most previous experiments eliminated this possibility. We suggest that the positive effects of dispersal on beta diversity, like the one we report here, may have been greatly underappreciated.

Keywords

Community assembly, microbial ecology, nectar sugar composition, null model, pollination, priority effect, species turnover.

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INTRODUCTION

Over the past decade, there has been a growing interest in understanding the determinants of beta diversity, the variation in species composition among local communities (Whittaker 1960), as a major component of biodiversity (Ricklefs 1987; Hubbell 2001; Condit *et al*. 2002; Anderson *et al*. 2011) that can influence ecosystem functioning (Pasari *et al*. 2013; Wang & Loreau 2014; Van Der Plas *et al*. 2016). One well-recognised determinant of beta diversity is dispersal of individuals among local communities. In the current literature, dispersal is almost invariably thought to homogenise communities, decreasing beta diversity (Loreau 2000; Mouquet & Loreau 2003; Cadotte *et al*. 2006).

There are three inter-related reasons for this expectation. First, a higher rate of dispersal may increase alpha diversity, the local species diversity, as a result of mass effects reducing the rate of local species extinction (Shmida & Wilson 1985). If gamma diversity, the overall regional diversity, was unaffected, an increase in alpha diversity would be automatically accompanied by a decrease in beta diversity as statistical inevitability (Chase & Myers 2011; Kraft *et al*. 2011; Lessard *et al*. 2012) (Fig. 1a). Second, increased dispersal may accelerate succession so that communities reach a late successional stage quickly (Vellend *et al*. 2014). As a result, when dispersal is uniformly high across communities, all communities likely reach a similar late stage, and their species composition will be similar (Fig. 1b). Third, by more quickly bringing all species, including poor dispersers, to local communities, increased dispersal may promote deterministic species sorting by competition and other local species interactions, resulting in strong convergence of communities in species composition. As a consequence, beta diversity is reduced (Fig. 1c).

Although rarely considered, there are also reasons to expect dispersal to increase, rather than decrease, beta diversity. First, increased dispersal may facilitate arrival of species that would not otherwise reach communities, effectively increasing gamma diversity (Vanschoenwinkel *et al*. 2013; Zobel 2016). If alpha diversity was not as affected by dispersal, beta diversity would be automatically increased (Driscoll & Lindenmayer 2009; Spasojevic *et al*. 2014) (Fig. 1d). Second, when increased from low to moderate rates, dispersal can increase variation among communities in their successional stage (Vellend *et al*. 2014). By definition, species composition changes as communities undergo succession. Thus, increased variation in successional stage should translate into increased beta diversity (Fig. 1e). Finally, if increased dispersal enhances the chance of arrival of species that would otherwise not be present, but would cause communities to diverge in species composition once colonised, the resulting divergence will contribute to beta diversity (Fig. 1f). The driver of such divergence is priority effects, in which the order of species arrival dictates the outcome of local competitive and other biotic interactions (De Meester *et al*. 2002; Fukami 2004; Steiner 2014).

Perhaps because it is intuitive to regard dispersal as a homogenising force that negatively affects beta diversity (Cadotte 2006; Soininen *et al*. 2007), little attention has been paid to the potential positive effects of dispersal outlined above. Empirical evidence available to date does appear to confirm the conventional intuition (e.g. Cottenie 2005). In reviewing metacommunity experiments, Grainger & Gilbert (2016) found that 80% of 26 studies that looked at the effect of dispersal on beta diversity reported a negative effect, 20% no significant effect, and none a positive effect. It is possible, however, that the widely accepted intuition led many authors to design experiments in ways that would detect a negative, but not positive, effect of dispersal. Furthermore, as Grainger and Gilbert (Grainger & Gilbert 2016) noted, many authors have manipulated dispersal using manual transfer or other methods that do not have a clear natural context. Field

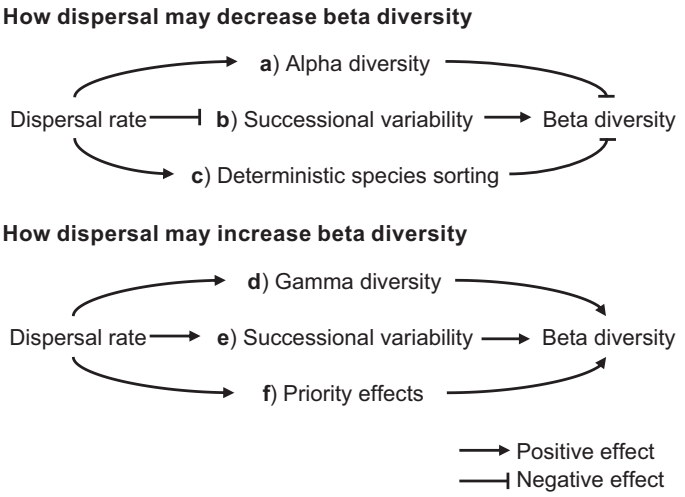

Figure 1 Hypotheses on how dispersal influences beta diversity.

experiments that directly reduce natural rates of dispersal and evaluate its effects on beta diversity are largely lacking (Kalamees & Zobel 2002; Zobel & Kalamees 2005), potentially distorting our understanding of factors driving beta diversity.

Here, we report the results of a field experiment that tested for the effects of naturally occurring dispersal on beta diversity. In this experiment, we reduced natural rates of dispersal by eliminating multiple modes of microbial dispersal. Specifically, we focused on nectar-inhabiting bacteria and yeasts that are dispersed among flowers by wind, insects, and birds (Brysch-Herzberg 2004; Belisle *et al*. 2012). We imposed increasing dispersal limitation on individual flowers and quantified microbial abundance, species composition, and microbial effects on nectar chemistry. Our focus in this study was dispersal, rather than environmental heterogeneity among local communities, which can contribute to beta diversity via species sorting (e.g. Cottenie 2005; Questad & Foster 2008). For this reason, we did this experiment with a single plant species to standardise for the level of inherent heterogeneity in the nectar environment across dispersal treatment groups. In stark contrast to findings from previous studies (reviewed by Cadotte 2006; Grainger & Gilbert 2016), we found that dispersal increased beta diversity, both taxonomically (species composition of microbes) and functionally (chemical composition of nectar). Our analyses suggested that the increased beta diversity was driven at least in part by priority effects (Fig. 1f), rather than merely reflecting statistical inevitability (Fig. 1d) or increased successional variability (Fig. 1e) alone.

MATERIALS AND METHODS

Study organisms

We conducted this study using microbial communities inhabiting the nectar of *Mimulus aurantiacus* Curtis (sticky monkeyflower, Phrymaceae), a native shrub that is common in chaparral and coastal scrub habitats throughout California and Oregon. At our study site located in the Jasper Ridge Biological Preserve (JRBP) in the Santa Cruz Mountains of California (37°408' N 122°2275' W), *M. aurantiacus* flowers are primarily pollinated by Anna's hummingbird (*Calypte anna*), although we also observe occasional visits by other hummingbird species (e.g. *Selasphorus rufus*), bees including *Bombus vosnesenskii*, *Ceratina acantha*, and *Xylocopa micans*, and thrips (Belisle *et al*. 2012). At JRBP, *M. aurantiacus* typically flowers from late April through early July, and its flowering phenology partially overlaps with that of a few other plant species in the chaparral habitats, including *Lepechinia calycina*, *Pedicularis densiflora*, and *Eriodictyon californicum*.

Bacteria and yeasts are known to inhabit floral nectar of many plant species (Brysch-Herzberg 2004; Herrera *et al*. 2008; Álvarez-Pérez *et al*. 2012; Fridman *et al*. 2012), including *M. aurantiacus* (Belisle *et al*. 2012; Peay *et al*. 2012; Vannette *et al*. 2013). These microbes are dispersed to flowers by pollinators and other floral visitors (de Vega & Herrera 2012) in addition to other potential means such as wind and rain water, and often attain high densities within floral nectar, for example, 10^4–10^5 colony forming units (CFU) per μL nectar (Herrera *et al*. 2008). Microbial activity in nectar can alter nectar chemistry (Herrera *et al*. 2008; Peay *et al*. 2012; Vannette *et al*. 2013) and influence plant-pollinator interactions (Herrera *et al*. 2013; Vannette *et al*. 2013; Schaeffer & Irwin 2014) by altering nectar chemistry (Good *et al*. 2014).

Experimental design

Within the same approximately 0.25-km^2 area at JRBP as used by Belisle *et al*. (2012), we chose 45 *M. aurantiacus* plants for experimental manipulation of flowers. On April 20, 2012, we caged some of the branches of all 45 plants using a cylindrical cage (approximately 40 cm in diameter and 80 cm height) covered with a black, plastic 2.5-cm mesh net, with a door that could open outward for researchers to access flowers inside the cage (Fig. 2a). The cages were placed so as to prevent hummingbirds from visiting flowers in them. We kept the cages on the plants throughout the *M. aurantiacus* flowering season, until July 2012. Three times during this period, in early May, early June, and late June, we placed a small jewellery tag that had a unique identification number written on it on each of six flower buds on each plant. Some of these buds were inside the cage, and others outside. We covered some of the tagged buds outside the cage in a small organza bag (Fig. 2a), in order to prevent not only hummingbirds, but also bees and other small flower-visiting animals from visiting the flowers.

Thus, we had three treatments to manipulate animal-assisted dispersal of microbes to flowers: (1) exposed flowers (no manipulation), open to microbial dispersal via any flower-visiting animal, (2) caged flowers, open to microbial dispersal via bees and other small animals, but not hummingbirds, and (3) bagged flowers, open to microbial dispersal only via wind and other means that do not involve an animal, although our observations in the field suggest that small thrips may have had occasional access to the bagged flowers. With this experimental design, we applied treatments haphazardly across plants and flowers to reduce any bias in environmental heterogeneity (e.g. inherent variation in the chemical properties of nectar) among treatments, allowing us to focus on the effect of dispersal on beta diversity (see Supplementary Methods

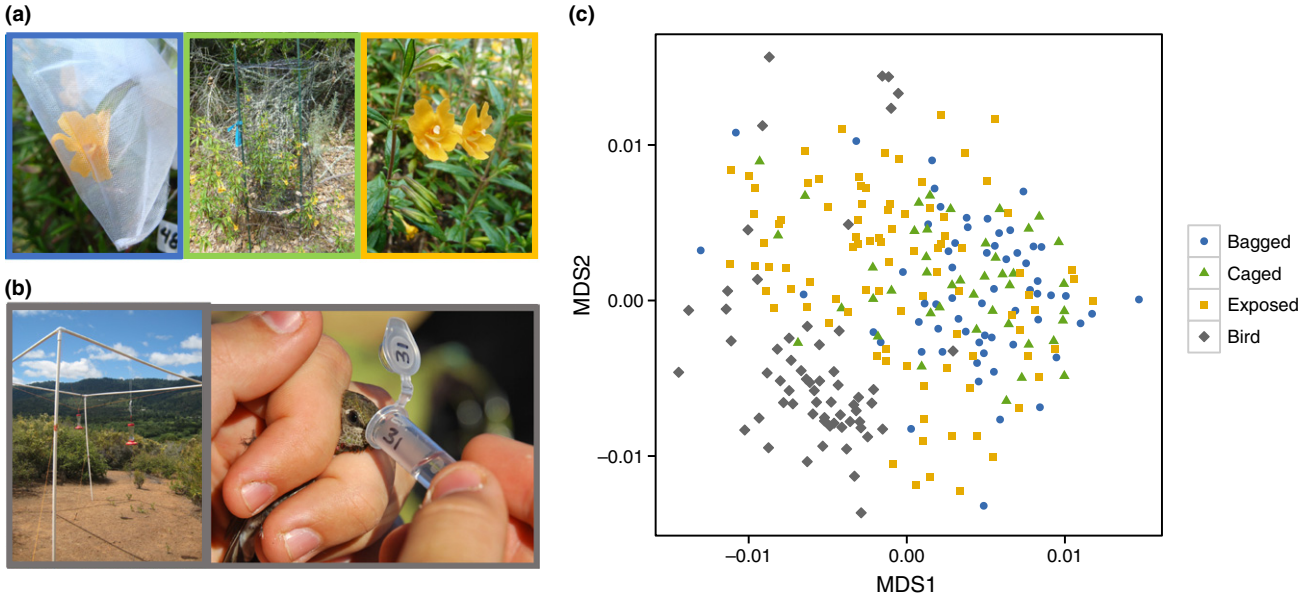

Figure 2 Field methods (a, b) and bacterial species composition in flowers and on birds (c). Panel a shows *Mimulus aurantiacus* flowers with varying dispersal treatments. Flowers were bagged using fine organza mesh designed to exclude all floral visitors (left image), caged using 2.5 cm mesh netting to exclude large-bodied pollinators, including hummingbirds (middle image), or exposed to all floral visitors (right image). Panel b shows hummingbird (*Calypte anna*) sampling methods. Birds were sampled by attracting birds to feeders and capturing by mist nets that were set up around the frame made of PCV pipes (left image). Captured birds were given a drink of sterile 15% sucrose solution (right image; photo credit: Nona Chiariello), and microbial communities from the remaining solution were characterised in the laboratory. Panel c shows the non-metric multidimensional scaling ordination of bacterial communities found in *M. aurantiacus* floral nectar or on *C. anna* hummingbird bills. Data points represent individual flowers or birds. The ordination was conducted using Bray-Curtis dissimilarities calculated from rarefied OTU tables. Bacterial taxa found only on bird bills (and not in nectar) were excluded from the NMDS analysis.

S1). A total of 348 nectar samples, including 104 bagged, 73 caged, and 171 exposed flowers, were recovered for analysis.

Each tagged flower bud was checked daily to record the date of anthesis (flower opening) and harvested 4 or 5 days after anthesis. While on the plant, flower stigma status (open or closed) was examined daily. Stigmas on *M. aurantiacus* close and do not re-open following pollination, which may require multiple pollinator visits (Fetscher & Kohn 1999). Thus, stigma status can be used as an indicator of pollinator visitation (Belisle *et al.* 2012; Peay *et al.* 2012).

To harvest flowers, we clipped them at the pedicel and stored them in a cooler until nectar could be extracted for microbial and chemical analysis. Cooler storage lasted no longer than 5 h. In the laboratory, the corolla tube was separated from the calyx, and nectar was collected from each flower using a 10- μL microcapillary tube. The volume of each sample was quantified, and nectar diluted in 30 μL of sterile water. Diluted nectar samples were used to estimate microbial abundance using colony forming units (CFUs), which is correlated with cell density in nectar (Peay *et al.* 2012). For CFUs, serial dilutions were plated on the day of harvest on yeast malt agar (YM) with 100 mg L^{-1} chloramphenicol (antibacterial) and on R2A plates supplemented with 20% sucrose and 100 mg L^{-1} cycloheximide (antifungal). Plates were incubated at 25 °C for 5 days, and colonies counted. The remaining diluted nectar samples were stored at −80 °C until immediately before we conducted DNA extraction for microbial species composition and UPLC-ELSD analysis for sugar composition, as described below.

We assessed if our dispersal treatments were effective in reducing visitation by floral visitors by using (1) logistic regression with dispersal treatments and sampling months as predictors of the frequency of flowers with closed stigmas as the response variable and (2) two-way ANOVA with dispersal treatments and sampling months as predictors of $\log_{10}$-transformed microbial abundance, separately for CFU data from YM and R2A plates. We found that bagged and caged flowers were, on average, 98 and 64% less likely to have a closed stigma than exposed flowers (exposed = 74.6%; caged = 26%, bagged = 1.5%), respectively ($\chi^2(2) = 83.79$, $P < 0.001$), and had 90 and 45% reduction, on average, in log-transformed microbial densities in nectar ($F_{2,274} = 3.05$, $P = 0.04$ for YM; $F_{2,278} = 3.35$, $P = 0.03$ for R2A, Fig. S1), indicating that dispersal treatments were effective in manipulating pollinator visitation and associated microbial dispersal.

Bird sampling

To verify that bacteria and yeasts found in flowers were vectored by pollinators, we caught hummingbirds for microbial sampling in late June 2012. Four mist-net stations with feeders containing 15% sucrose were established within the area in which the 45 *M. aurantiacus* plants used for the experiment were located (Fig. 2b). We chose to mist-net birds only at the end of microbial sampling from flowers in order to minimise the effect of the presence of feeders on microbial distribution among flowers and bird behaviour during the sampling period. Captured birds were allowed to drink sterile sugar water

(2 mL of 20% sucrose was provided), immediately after which the birds were banded and released. An aliquot of each remaining sugar water sample (approximately 1.5 mL) was processed in the laboratory as described below. Over three consecutive days of mist netting, 61 samples were recovered from a total of 48 individual *C. anna* hummingbirds (some birds were caught multiple times).

DNA extraction, PCR, sequencing, and bioinformatics

We extracted DNA from 10 μL of each diluted sample for amplification of bacterial and fungal ribosomal rRNA gene (White *et al.* 1990). Samples were then sequenced (Caporaso *et al.* 2010; Smith & Peay 2014) and analysed (Wang *et al.* 2007; Joshi & Fass 2011; McMurdie & Holmes 2013; Peay *et al.* 2013; Edgar 2013, Deshpande *et al.* 2015; Weiss *et al.* 2015) to obtain rarefied tables of operational taxonomic units (OTUs). See Supplementary Methods S2 and Fig. S2 for detail.

Chemical analysis of nectar

Sugar composition was quantified within individual nectar samples. Samples were further diluted (approximately 1 : 10) and sugars were separated using UPLC and quantified using ELSD and a series of external standards, following the methods described in Vannette & Fukami (2014). The proportion of monosaccharides in nectar, that is, (glucose + fructose)/(glucose + fructose + sucrose), was calculated and used as a response variable. We focus on this proportion because of its functional importance to pollinator visits: sugar composition, particularly the proportion of monosaccharides, has been shown to influence foraging decisions by birds (Martínez del Rio *et al.* 1992).

Statistical analysis of species composition

To visualise the similarity of microbial communities based on their relative abundance in individual nectar and bird samples, we used NMDS to ordinate samples based on Bray-Curtis dissimilarity matrices using the R package vegan (Oksanen *et al.* 2015), separately for bacteria and fungi. NMDS was chosen because it allows for visualisation of distance among samples in a few dimensions (Legendre & Legendre 1998). Qualitatively similar results were obtained using other dissimilarity matrices (Fig. S3). We assessed whether the similarity in microbial community structure between flower and bird communities differed among the dispersal treatment groups by (1) calculating all possible pairwise distances between the community in a nectar sample and the community on a bird in the NMDS space and (2) comparing these distances among the dispersal treatment groups (caged, exposed, or bagged) using ANOVA. Calculations were repeated using the distance from each flower to the nearest bird sample in NMDS space, which yielded a result qualitatively the same as for the all possible pairwise comparisons.

Statistical analysis of species diversity

To compare beta diversity among the three dispersal treatments, we quantified variation in community composition within each dispersal treatment, measured by the distance to the centroid of each treatment group (Anderson *et al.* 2006), using the betadisper function in the vegan package (Oksanen *et al.* 2015). This analysis tests for differences in variance in species composition among treatment groups.

To control for variation in gamma diversity and the number of samples in each treatment group, we calculated beta diversity using the multivariate method based on dissimilarity matrices (Anderson *et al.* 2011; Legendre & Cáceres 2013). Briefly, samples were split into the dispersal treatment groups. For each sample within each treatment, the distance to the treatment centroid (calculated using the betadisper function) was extracted. We compared the observed beta diversity measures to null expectations using a modified version of the method developed by Kraft *et al.* (2011), Myers *et al.* (2013), and Tello *et al.* (2015). Specifically, within each dispersal treatment, null communities were generated by subsampling with fixed regional species abundance distributions, following Tello *et al.* (2015). For empirical and null communities separately, the distance to the treatment centroid was then extracted, with the number of points equivalent to that in the corresponding treatment group. We subtracted each empirical distance from the corresponding null distance and divided it by the SD of the null community distances. These measures of the difference between observed and expected beta diversity are termed beta deviation (see details and R code in Appendix S1). Note that our beta deviation, which is calculated for each replicate community, is different from Tello *et al.*'s (2015), which computes a single measure of beta deviation per treatment. We used the modified, per-replicate calculation because it enables statistical tests of treatment effects on beta deviation. Either way, beta deviation is used for comparison of the observed level of beta diversity in each treatment to those expected if the communities were assembled randomly within the constraints imposed by the observed level of species abundance distributions within the treatment (Tello *et al.* 2015).

If communities were assembled randomly, beta deviation would be statistically indistinguishable from zero. On the other hand, if communities diverge more than expected by chance, beta deviation will be higher than zero. We calculated and compared beta deviation among treatments using ANOVA and TukeyHSD *post hoc* tests (see details and R code in Appendix S1) using linear and mixed models that included plant identity as a random effect. Finally, because null model approaches may be subject to variation in species abundance distribution or sample coverage, we also performed the pairwise analysis of beta diversity as described by Bennett & Gilbert (2016).

In addition, because flowers could not be sampled from all dispersal treatments in late June owing to limited flower availability during the late season, we re-ran all above analyses without the data from late June to verify that the dispersal treatment effects identified were not confounded by the biased sampling in late June.

As described above, experimentally imposed limitation of microbial dispersal reduced microbial colonisation, with bagged, caged, and exposed flowers ranked in that order in both

closed stigma frequency (an indicator of microbial dispersal) and microbial abundance, suggesting that increased dispersal accelerated microbial succession toward high total abundance. For this reason, we sought to test if the difference in microbial density (which was highly correlated with abundance) influenced beta diversity. We did this test by grouping flowers into three bins according to CFU density ($0–10^1$, $10^1–10^3$, and $10^3–10^6$ CFU per μL + 1 for R2A plates and $0–10^1$ and $10^1–10^6$ CFU per μL + 1 for YM plates), repeating the null model analysis within each bin, and comparing beta diversity among the density-based groups of flowers using ANOVA and TukeyHSD tests. We also analysed the data with Kruskall–Wallis rank sum tests to verify that observed differences were robust to assumptions made by parametric tests used.

In addition to analysing beta diversity, we also compared the dispersal treatment groups in alpha diversity (rarefied OTU richness) using ANOVA as well as in estimated gamma diversity using unconditional estimates and 95% confidence intervals (Colwell *et al*. 2004, 2012). We defined gamma diversity as the total observed diversity in all flowers belonging to the same dispersal treatment group, rather than overall diversity of all treatments taken together.

Statistical analysis of nectar chemistry

The nectar of *M. aurantiacus* is typically 25–30% sugar (w/v). In the absence of microorganisms, approximately 80% of this nectar sugar is sucrose, with the rest being glucose and fructose (Vannette *et al*. 2013). Invertases produced by yeasts and bacteria would hydrolyse sucrose to its component monosaccharides, glucose and fructose. We used the proportion of monosaccharide sugars (glucose and fructose) within nectar samples as a measure of microbial effects on the functional characteristics of nectar that have been shown to modify pollinator preference (Canto & Herrera 2012; Vannette *et al*. 2013; Vannette & Fukami 2016). We used multiple regression to examine the effects of microbial density (CFU data from YM and R2A plates) and species composition on the proportion of monosaccharide sugars in nectar. Species composition was represented by the first three Principal Coordinate Analysis axes summarising bacterial species composition based on Bray-Curtis dissimilarity, which is linearly related to distance among samples, as opposed to NMDS axes used previously (Ramette 2007). Stepwise regression based on AIC was used for model simplification. Mixed models were run using lme4 (Bates *et al*. 2015), with plant identity as a random effect, and marginal R^2 values were calculated following Nakagawa & Schielzeth (2013). We also used Levene's test to determine if variance in the proportion of monosaccharide sugars differed among dispersal treatments.

RESULTS

Species composition

Bacterial composition differed among dispersal treatments (Fig. 2c, PERMANOVA $F_{2,188} = 7.24$, $P < 0.001$ $R^2 = 0.07$), whether or not samples from late June were omitted from analysis (Dispersal $F_{2,168} = 7.43$, $P < 0.001$; Table S1). We found qualitatively identical patterns using other dissimilarity measures, including Jaccard, UniFrac, and other methods of visualisation (Fig. S3). Fungal composition varied primarily with sampling month (PERMANOVA $F_{2,180} = 8.30$, $P < 0.001$), with abundance increasing from May to late June (Fig. S1b), but when late June samples were omitted, fungal composition also differed among dispersal treatments (Dispersal $F_{2,150} = 1.72$, $P = 0.04$, Fig. S4). Microbial composition on birds was variable among bird individuals (Fig. 2c, Fig. S5), but the taxa that were abundant in nectar, such as *Acinetobacter* sp. and *Neokomagataea* sp. for bacteria and *Metschnikowia reukaufii*, *Fusarium* spp., and *Aureobasidium pullulans* for fungi, were also frequently found on birds (Fig. S5). We also found that exposed flowers were more similar to bird samples compared to bagged and caged flowers in bacterial composition ($P < 0.001$; Fig. 2c, Fig. S3).

Species diversity

The ordination plots indicated that exposed flowers were more variable in bacterial composition than bagged and caged flowers (Fig. 2c, Fig. S3). This trend was confirmed by the analysis of beta diversity, which was nearly twice as high among exposed flowers as among bagged or caged flowers (Fig. 3a, Table S2). Beta deviations calculated using null model analysis also indicated that communities in exposed flowers were more variable than by chance alone and 1.5–2 times as variable as those in caged and bagged flowers (Fig. 3b; Table S3). These results were consistent with the full dataset and when late June samples were omitted ($P < 0.001$ in both cases). The complementary metric, pairwise dissimilarity (Bennett & Gilbert 2016), also indicated that flowers exposed to pollinators had a higher average dissimilarity between samples than those from caged and bagged treatments (Tukey HSD $P < 0.0001$ for all comparisons; Fig S6). Fungal composition varied primarily among months ($P < 0.001$), but with late June samples omitted, exposed flowers had higher fungal beta diversity than caged flowers (beta diversity $P = 0.04$, Fig. S7), though not higher than bagged flowers.

When grouped by CFU density, communities with higher densities had higher beta diversity in both bacteria (Fig. 4) and fungi (Fig. S8). Results were robust to ±0.5 log units for each of the middle cutoff bin values.

Despite differences in CFU density among dispersal treatments (Fig. S1), alpha diversity within individual flowers did not vary significantly among the treatments (Fig. S9a, $P = 0.23$). Sampling curves for gamma diversity had begun to saturate, and gamma diversity in exposed flowers was significantly greater than in bagged flowers, with caged flowers of intermediate diversity ($P < 0.05$, Fig. S9b).

Nectar chemistry

The proportion of sugars comprised by glucose and fructose varied among dispersal treatments (Fig. 5a). Specifically, the average proportion of monosaccharides in exposed flowers was approximately double that of bagged flowers. Sugar composition was also associated with bacterial species composition (bacterial principal component 3, Fig. 5b) and the

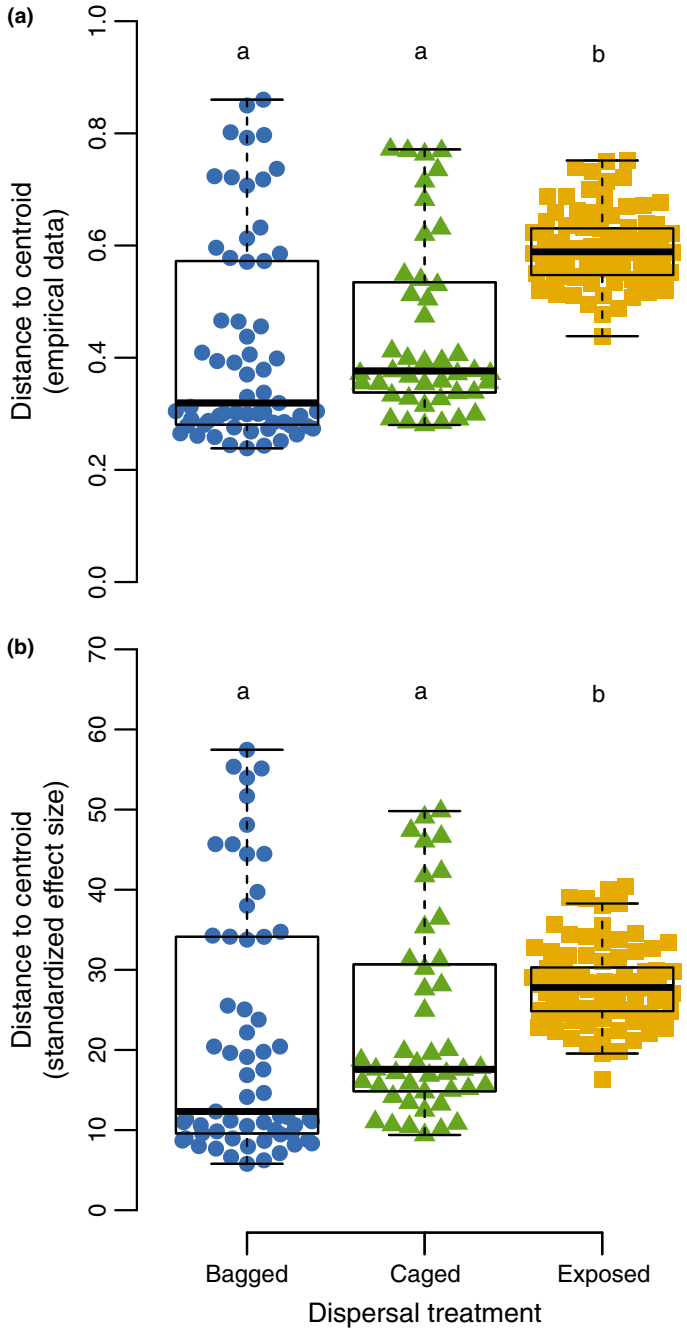

Figure 3 Effect of floral dispersal treatments on β diversity in bacterial communities. Distance to centroid in the empirical data in panel (a) was calculated in multivariate space using betadisper. Distance to centroid in panel (b) represents the standardized difference between distance to centroid calculated in the empirical and null communities, called beta deviation throughout the manuscript (see Methods and Appendix for further details). Null model analyses were conducted as described in the Methods section using 999 permutations. Nectar samples from all months are included in both panels. Letters indicate treatments that differ significantly at $P < 0.01$, using a Tukey HSD test. Boxplot hinges represent first and third quartiles, error bars indicate 95% confidence intervals, and bar in each box represents the median.

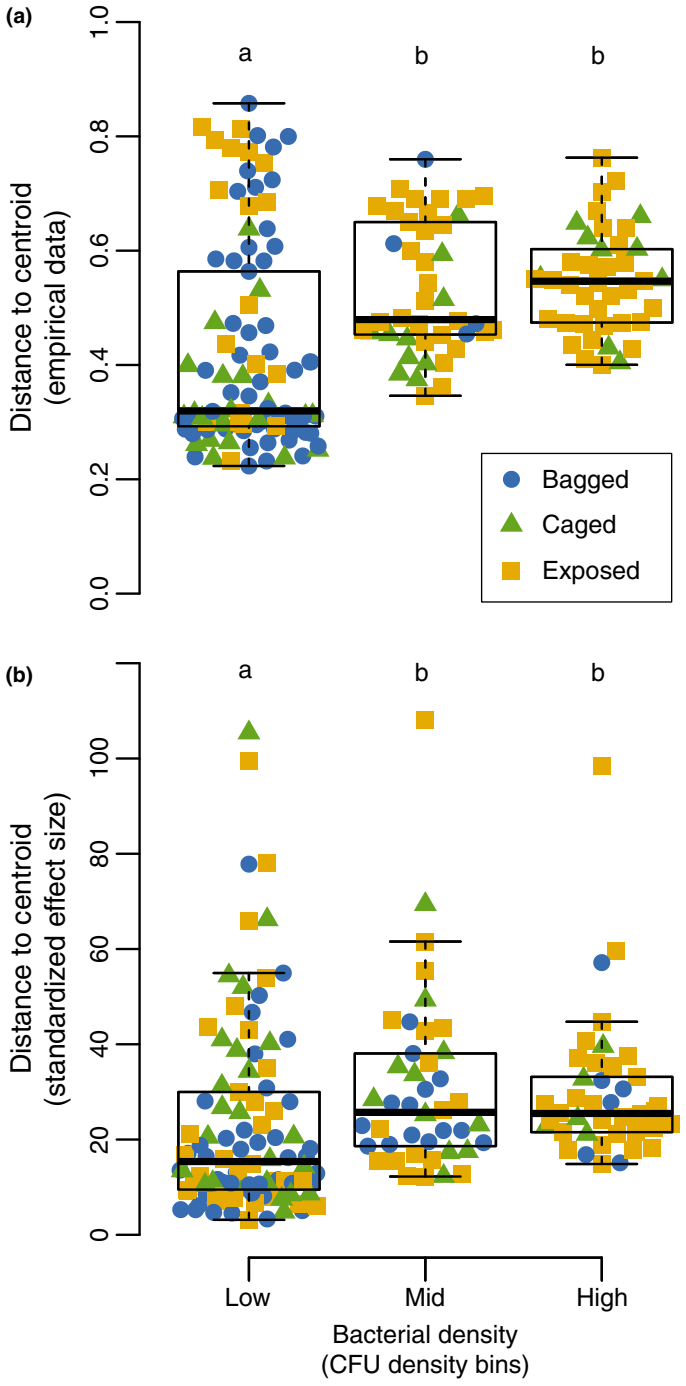

Figure 4 Relationship between bacterial colony-forming unit (CFU) density in nectar of *Mimulus aurantiacus* and (a) β diversity estimated from bacterial empirical data (b) β deviation from null model predictions. Letters indicate treatments that differ significantly at $P < 0.01$, using a Tukey HSD test. Boxplot hinges represent first and third quartiles, error bars indicate 95% confidence intervals, and bar represents the median.

density of both bacteria and yeasts in nectar (Fig. 5c, d). Together, these four predictors (dispersal treatment, bacterial PC3, bacterial density, fungal density) explained 49% of the variation in composition of mono- and disaccharides among flowers. Similar to bacterial composition (Fig. 3), sugar composition was more variable among exposed flowers than among bagged or caged flowers (Fig. 5a, Levene's F $P < 0.001$).

DISCUSSION

Contrary to the conventional theory (Mouquet & Loreau 2003) and overwhelming evidence (Grainger & Gilbert 2016; Catano *et al*. 2017) that dispersal decreases beta diversity, we found here that beta diversity was highest among communities that were allowed the greatest potential for dispersal (Figs 2c and 3a). Furthermore, we also found that dispersal not only enhanced beta diversity, but also increased variability in a functional property of flowers that the nectar microbes modify, that is, sugar composition of nectar (Fig. 5).

Multiple mechanisms might explain this result, but the null-model analyses indicated that statistical inevitability (Fig. 1d) alone would not suffice to explain it (Fig. 3b). An alternative explanation involves successional variability (Fig. 1e). That is, because not every flower was pollinated, even when exposed to pollinators, the high beta diversity among exposed flowers could have been driven simply by a large disparity between exposed flowers that were pollinated vs. non-pollinated. However, beta diversity was higher for flowers with higher microbial abundance (Fig. 4a-b), even when only pollinated flowers were considered ($P < 0.01$), which refutes the argument that a large disparity in pollination status—and the associated variation in the successional stage of microbial communities as reflected in their total abundance—fully explains the higher beta diversity in exposed flowers than in bagged and caged flowers (Fig. 1e). In other words, we observed higher variability among communities with a high density of microbes, suggesting that divergent community states were generated by biotic interactions rather than simply by variance due to the presence and absence of dominant microorganisms. Moreover, we can attribute the increased beta diversity largely to dispersal via hummingbirds because exposed flowers had greater beta diversity than caged flowers, while caged flowers did not differ from bagged flowers in most analyses.

The increased level of community divergence in the relatively homogenous nectar environment of a single host plant species, particularly at high microbial densities (Fig. 4), suggests that historically contingent competitive exclusion or other species interactions contributed to community dissimilarity. Specifically, increased beta diversity was not simply the result of greater gamma diversity in flowers exposed to pollinators. Together, these data point to the possibility that priority effects contributed to the observed patterns of beta diversity (Fig. 1f). Although we did not directly test for priority effects in this study, laboratory and field experiments have shown strong priority effects in nectar bacteria and yeasts (Peay *et al*. 2012; Tucker & Fukami 2014; Vannette & Fukami 2014; Toju *et al*. in revision). More research is needed to directly test for the effect of priority effects on beta diversity in naturally occurring flowers in the field.

The specific mechanisms of increased beta diversity remain unclear, but five lines of evidence suggest that competitive interactions between at least two bacterial taxa, *Acinetobacter* and *Neokomagataea*, may have been primary drivers of community divergence via priority effects. First, these taxa were commonly found and had a higher incidence and average abundance in nectar when dispersal was not experimentally limited (Fig. S10). Second, *Acinetobacter* and *Neokomagataea* were differentially associated with major axes of variation in bacterial composition in flowers (Fig. S11) and often prevalent when bacterial density was high. Third, these taxa were present on nearly all hummingbird bills, but their relative abundance varied greatly among bird individuals (Fig. S5), making it likely that the two bacterial taxa's arrival order, or at least initial abundance, relative to each other varied greatly among exposed flowers. Fourth, the number of bacterial cells isolated from hummingbird bills was at least an order of magnitude less than in nectar, suggesting that mass effects were unlikely to determine patterns of beta diversity. Finally, a recent field study in *M. aurantiacus* suggested long-term priority effects involving these species: experimental inoculation of flowers with *Neokomagataea* sp. led to exclusion of Gammaproteobacteria (primarily *Acinetobacter* spp.) from nectar (Toju *et al*., in revision).

To more definitively test for the effect of dispersal rate on the contribution of priority effects to beta diversity, future research should directly manipulate microbial arrival order and quantify its effects on microbial and sugar composition in

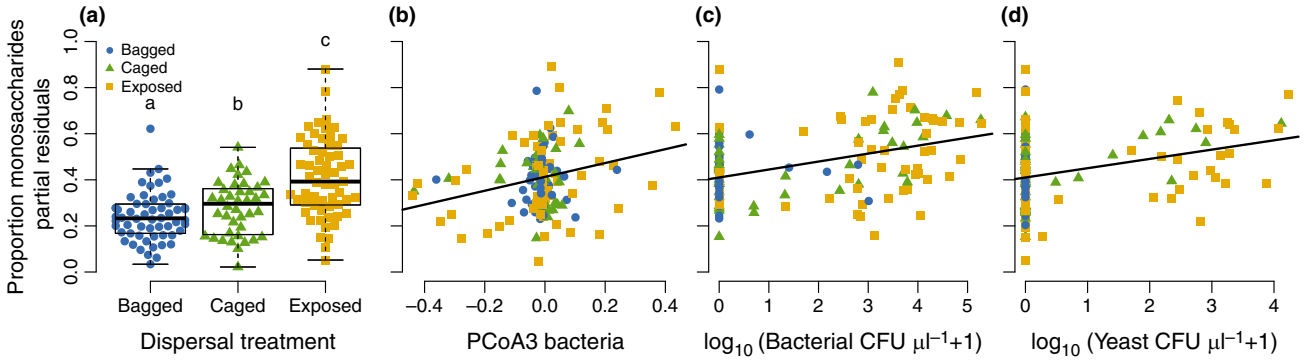

Figure 5 Results of multiple regression examining microbial predictors of nectar sugar composition, as measured by the proportion of sugars comprised of monosaccharides. Panels show the effects of individual predictors that were retained following model selection. Conditional plots (Breheny & Burchett 2013) are shown where partial residuals were plotted against the focal predictor while controlling for the other predictors in the model. Retained predictors included: (a) dispersal treatment, (b) PCoA axis 3 (6% of total variance in bacterial community composition explained), (c) $\log_{10}$(bacterial CFU per µL + 1) in floral nectar, and (d) $\log_{10}$(fungal CFU per µL + 1) in floral nectar. All predictors are significant at $P < 0.01$. Letters indicate results of Tukey HSD test. See Fig. S11 for correlations between individual taxa and PC axis 3. Boxplot hinges represent first and third quartiles, error bars indicate 95% confidence intervals, and bar represents the median.

nectar. This work should be done with flowers in the field using experimental designs similar to the laboratory experiments that yielded evidence for pervasive strong priority effects in these microbes (Peay *et al*. 2012; Tucker & Fukami 2014; Vannette & Fukami 2014; Pu & Jiang 2015; Mittelbach *et al*. 2016). *Acinetobacter* and *Neokomagataea* should be reasonable initial species to focus on for arrival order manipulations. These field experiments could be particularly revealing if arrival order manipulations and dispersal treatments were implemented in a factorial fashion. As a complementary approach, sampling flowers of varying ages and directly quantifying pollinator visitation rates should also improve our understanding of microbial community assembly and its consequences for beta diversity.

More generally, does our finding of dispersal increasing beta diversity reflect a peculiar dispersal ecology of nectar microbes, or does it rather represent an overlooked phenomenon that may prove common across many types of microbial, plant, and animal communities? Only more empirical data can answer this question, but we suggest that the role of priority effects may have often been left unnoticed in previous dispersal experiments owing to unrealistic manipulations of dispersal. In many experiments, dispersal is manipulated by simultaneous bulk propagule addition (reviewed by Cadotte *et al*. 2006; Myers & Harms 2009; Grainger & Gilbert 2016), which limits the scope for priority effects (but see Pu & Jiang 2015). In natural systems, stochastic variation among local communities in the timing of dispersal, and therefore arrival order, is common, which would allow the mechanisms implicated here to generate priority effects (Fukami 2015; Grainger & Gilbert 2016). On the other hand, at very high dispersal levels, mass effects or source-sink dynamics may preclude priority effects (Chase 2003; Fukami 2015). One logical expectation may then be that beta diversity first increases, in part due to stronger priority effects, but then declines, in part because of mass effects, with increased dispersal rate. Testing this prediction would require multiple levels of dispersal limitation to be manipulated, as in this study, and these tests should be more informative if dispersal was manipulated in a way that allows the role of realistic dispersal to be evaluated.

CONCLUSION

We have used our experimental results to argue that dispersal can promote beta diversity via priority effects. We did this experiment with nectar microbes because of the ease with which the level of natural dispersal could be manipulated, but one common characteristic of natural dispersal across a variety of microbial, plant, and animal communities, not just in nectar microbes, is that dispersal history is largely stochastic and can thus be highly variable among communities. Our results extend knowledge of the relationship between dispersal and beta diversity, suggesting that increased dispersal can contribute to community divergence under some circumstances. Because stochastic dispersal has been purposefully eliminated in most previous experiments, the positive effects of dispersal on beta diversity, like the ones we have reported here, may be more prevalent than currently thought.

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AUTHORSHIP

RLV and TF conceived the study, conducted field work, and designed data analysis. RLV conducted lab work and analysed data. Both authors contributed to writing.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.75b30 and NCBI SRA under BioProject PRJNA385866.

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