Title: Fungi are more dispersal-limited than bacteria among flowers

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Abstract:

Dispersal, particularly variation in dispersal ability among taxa, affects community assembly in individual communities and biodiversity maintenance within metacommunities. Although fungi and bacteria frequently coexist, their relative dispersal abilities are poorly understood. Here, we compare the incidence and abundance of culturable flower-inhabiting bacteria and fungi among individual flowers. Using collections that span two coflowering communities across two years, we assess viable bacterial and fungal incidence and abundance within individual flower samples, and examine patterns across plant species that differ in flower traits. Our results demonstrate that bacteria can be detected in more flowers and in greater numerical abundance than fungi, particularly in flowers with more exposed corollas. For fungi, however, flowers with long corollas were equally likely as exposed flowers to contain cells, and hosted higher numbers of fungal cells, primarily yeasts. Across all flowers, bacteria and fungal incidence was positively related, but within flowers containing microbes, bacterial and fungal incidence was negatively related, suggesting shared dispersal routes but competition among microbes within flowers. The difference in dispersal abilities of bacteria and fungi identified here may have broad relevance for community assembly of microbes and plant-pollinator interactions.

Introduction

All organisms exhibit some degree of dispersal limitation. Variation in dispersal ability among species can have consequences for the composition and function of individual communities and biodiversity maintenance within metacommunities (Leibold et al. 2004). Dispersal limitation is well-documented for macro-organisms, and microbial taxa can also be dispersal limited (Peay et al. 2012). Despite the fact that bacteria and fungi often co-occur within habitats, few studies have examined their relative dispersal abilities. In some cases, bacteria and fungi can share modes of long-distance or even short-distance dispersal (Barberán et al. 2015). However, comparisons in dispersal ability between bacteria and fungi are rare and limit the ability to predict the relative importance of dispersal for these groups of organisms, or consequences for community patterns. For example, if bacteria and fungi are similarly dispersal limited, interactions within local communities may be most important in determining species composition, but if dispersal limitation differs between bacteria and fungi, metacommunity dynamics may be more important. In the second case, if bacteria are better dispersers but fungi are more dominant competitors, competition-colonization dynamics could be predicted. However, processes underlying microbial community assembly and dynamics likely differ from those structuring communities of macroorganisms (Koskella et al. 2017).

Bacteria and fungi often occur in nectar of flowers (Herrera et al. 2009, Fridman et al. 2012). Nectar-inhabiting microbial communities are ideal for examining dispersal limitation for a few reasons. First, communities in nectar undergo primary succession. Few culturable microbes are found in nectar of newly opened flowers that have not received visitation by animals (Vannette and Fukami 2017, von Arx et al. 2019), but flower visitors introduce bacteria and fungi that successfully colonize floral nectar (Lachance et al. 2001, Herrera et al. 2008). Second, the majority of bacteria and fungi that survive and thrive in floral nectar are largely culturable (Morris et al. 2020)— shotgun sequencing of nectar microbial communities reveal qualitatively similar taxonomic composition to culture-based analyses. Third, the bacteria and fungi that grow in nectar are a small subset of the microbial communities introduced to nectar (Herrera et al. 2010, Belisle et al. 2012, Alvarez-Pérez and Herrera 2013), exhibit traits that may enhance fitness in nectar environments (Álvarez-Pérez et al. 2012, Pozo et al. 2012a, Dhami et al. 2016), and predominately depend on phoresy via flower visitors (Brysch-Herzberg 2004, Vannette and

Fukami 2017, Morris et al. 2020). Finally, laboratory experiments and field observations suggest that inhibitory priority effects influence community structure in floral nectar (Tucker and Fukami 2014, Toju et al. 2018). However, positive interactions among nectar-inhabiting taxa have been suggested (Alvarez-Pérez and Herrera 2013), so the relative influence of dispersal and species interactions (including competition) on nectar microbe metacommunities are not clear. Elucidating patterns of bacteria and fungi dispersal limitation in nectar would shed light on mechanisms underlying their community assembly, interactions between these groups in nectar, and their ecological relevance for plants and floral visitors.

Here, we test the hypothesis that bacteria and fungi differ in dispersal limitation among individual flowers in two coflowering communities. We examined bacterial and fungal occupancy (incidence) and abundance in individual nectar samples of more than 1800 flowers, spanning 43 species of plants at two sites to examine 1) do bacterial or fungal incidence and abundance vary among plant species? 2) Is observed variation associated with floral traits? 3) Is variation in nectar volume and sugar concentration and composition associated with variation in fungal or bacterial abundance?

We found that bacteria occupy a higher proportion of flowers than do fungi, and flowers with more accessible morphology (e.g. exposed nectary) more frequently contain detectable microbes. Moreover, abundant fungal populations are mainly restricted to flowers with long corollas. The incidence of both bacteria and fungi were associated with lower nectar volumes, indicating the importance of dispersal agents for microbial inoculation. However, in flowers containing microbes, bacterial and fungal abundance were positively associated with nectar volume, suggesting that time since previous visitation affects microbial abundance in nectar. Patterns of co-occurrence in flowers suggest that bacteria and fungi shared dispersal agents, but negative associations within individual flowers also suggest competitive interactions within nectar. These results suggest not only that bacteria and fungi differ in dispersal ability among flowers, but suggest the possibility that competition-colonization dynamics could be important in community membership and effects on plant-pollinator interactions.

Materials and methods

Field sites and flower sampling

During the peak flowering seasons of 2016 and 2017, standing crop floral nectar was sampled from two sites in northern California: Stebbins Cold Canyon Reserve (Stebbins) in Winters, CA and at flowering plots maintained at the Laidlaw Honey Bee Facility (Bee Biology) in Davis, CA. The sites are approximately 18 miles apart, so are unlikely to be linked by pollinator dispersal, and differ in pollinator species composition and anthropogenic influence, but share a subset of plant species.

Between late February and early July, flowers were sampled every two-four weeks from available plant species, with approximately 10 flowers of each plant collected per week (collections were limited by floral availability and reserve collection restrictions to protect plant populations). When possible, flowers were sampled from multiple individuals and sub-populations or plots (Supplementary Table S1). Care was taken to sample flowers that had been open at least one day if possible, to allow the opportunity for floral visitation and microbial immigration to flowers. Individual inflorescences were collected, placed upright in humidified boxes and kept cool until extraction and plating, no more than 5 hours later. In the lab, flowers were destructively sampled. Nectar was collected using 10 µl microcapillary tubes, and volume quantified. Nectar was diluted in 30 µl of sterile water (D0), then diluted 10 and 100 fold (D1 and D2 respectively) in sterile phosphate-buffered saline. To assess fungal and bacterial abundance, 50 µl of the 10 fold dilution (containing 5 µl of D0) and 50 µl of the 100 fold dilution (containing 0.5 µl of D0) were plated on yeast media agar (YMA) containing chloramphenicol and Reasoner's agar containing cycloheximide (R2A Oxoid formula with 20% sucrose), respectively. All samples were plated the day of collection. For convenience, we refer to the total number of colonies on YMA as "fungi" and colonies on R2A as "bacteria" throughout the manuscript although some colonies on each media type may be comprised by microbes resistant to the antimicrobial compounds used here (e.g. bacteria resistant to chloramphenicol (Dhami et al. 2018)). The threshold for detection was approximately 8 live cells for YMA media and 60 cells for R2A media in the original nectar sample. Negative controls were included and plated to detect potential contamination and samples discarded if contamination was detected (detected on 1 date; these samples were removed from the analysis). Agar plates were incubated at 28^{o}C and colony-forming units (CFUs) counted after 48-72 hours.

The total number of CFUs and CFU density for each nectar sample was calculated based on dilutions and original sample volume. Over the course of the study, 1 825 nectar samples were collected and plated on two media types. In 2016, representative colonies were picked haphazardly from plates collected over the entire season including all sites and plant species and frozen in glycerol.

A subset of microbial strains from glycerol stocks were identified using MALDI-TOF and spectra were compared to Bruker Bacteria and Eukaryote libraries and a custom in-house database curated from previously identified microbial isolates from nectar (Supplementary Methods 1).

Floral traits

To examine if bacterial or fungal abundance differed between plant species whose flowers differed in morphology (likely influencing dispersal rate or frequency), we classified plant species based on corolla length which often limits accessibility of nectar to floral visitors (Kingston and Mc Quillan 2000, Lara and Ornelas 2001). Categories included “short” (1-5 mm), “mid” (5-15 mm), or “long” (15+ mm) corolla based on corolla tube length. Corolla color (red, blue/purple, white/light, yellow) was also recorded.

Sugar analysis

To examine if bacterial or fungal abundance was associated with nectar sugar concentration or composition, we quantified sugars in nectar samples. Only samples collected in 2016 were analyzed (N=764), but represented both sites and one entire flowering season. For each sample, 5 µl of diluted nectar (D0) was added to 45 µl of 50:50 Acetonitrile: water containing a 0.5 mg/ml of maltose as an internal standard. Each 2 µl injection was separated using a Thermo (Dionex) binary pump HPLC system on a Thermo Accucore 150-Amide-HILIC column (2.6um; 50 x 2.1mm I.D) at 0.17 mL/min using a 5 minute gradient starting at 80:20 Acetonitrile:water, decreasing linearly to 50:50, followed by a 10 minute equilibration at initial conditions. Saccharides were quantified using charged aerosol detection. Peak areas were quantified for mono- and disaccharides, and a series of external standards was used to calculate sugar concentration in each sample.

Statistical analysis

To determine if bacteria and fungi differ in incidence in floral nectar, across years or between sites, or by plant species, we used a logistic regression implemented using 'glm', using microbe (bacteria or fungi), site, year and plant species, and their two-way interactions as predictors. Significance was assessed using likelihood ratio tests. To determine if bacteria and fungi differ in abundance in floral nectar across years or sites, or by plant species, we used a linear regression implemented using 'lm', with log10(microbe CFUs+1) as a response and microbe (bacteria or fungi), site, year and plant species, and their two-way interactions as predictors. Interactions were dropped from the model if not significant. Significance was assessed using F-tests. To compare if floral traits (corolla length or flower color) was associated with bacterial or fungal presence or abundance, we used logistic regression and linear regression as above, but replaced plant species with color and nectary location (single model), and included all two-way interactions. To examine the relationship between bacterial and fungal incidence in individual nectar samples, we used Pearson correlations and repeated this analysis including only flowers that contained detectable microbes. Within flowers that contained microbes, we also assessed if bacterial and fungal abundance were associated using Pearson correlations of log-transformed CFU counts. To examine if nectar characteristics were associated with microbial incidence or abundance, we used linear regression with log10(nectar volume(µl)+0.1) as the response variable and plant species, microbial incidence and abundance, and site as predictors. This analysis was repeated for total sugar concentration and the proportion of nectar sugars comprised by monosaccharides but the HPLC run was included as a random effect and nectar volume was included as a covariate. Mixed effects models were performed using lme4 (Bates et al. 2011). Significance of factors was assessed using F-tests and of continuous variables using t-tests to examine directionality of effects. All analyses were conducted using RStudio and R v. 3.6.3.

Results

Patterns of bacterial and fungal incidence

Detectable colonies on R2A media (hereafter 'bacteria') were isolated from nectar more frequently than were colonies on YMA (hereafter, 'fungi'): bacteria were detected in 49% of samples but fungi in only 20% of samples (microbe LRT=21.07, $P<0.001$, Fig. 1). Microbial

incidence differed between geographic sites, microbes were found in nectar more frequently at Bee Biology than at Stebbins Reserve (54% vs 46%, LRT = 14.5 $P<0.001$, Supplementary Fig. S1a). Microbial incidence differed between years (LRT= 19.6, $P<0.001$) and among plant species (LRT = 293.8, $P<0.001$). Bacteria and fungi differed in patterns of incidence among sites and plant species. In contrast to bacteria, fungi were more likely to occur in flowers at Stebbins than at Bee Biology (microbe x site LRT= 4.3, $P=0.03$), and differed in patterns of incidence among plant species compared to bacteria (microbe x plant LRT=81.1, $P<0.001$).

Patterns of bacterial and fungal abundance in nectar

Within flowers that contained microbes, bacteria were more abundant than fungi, averaging at least an order of magnitude greater than fungal CFUs (microbe $F_{1,1904}=112.8$, $P<0.001$) despite a higher detection threshold for bacteria. Microbial abundance was greater at Stebbins Reserve than at Bee Biology for both bacteria and fungi (site $F_{1, 1904}=5.6$, $P=0.02$, Supplementary Fig. S1b). Microbial abundance differed between years ($F_{1, 1904}=4.2$, $P=0.04$). Interactions between microbial identity (bacteria vs fungi) and site or year were not significant ($P>0.10$) so were dropped from the model. Microbial abundance varied widely among plant species ($F_{41, 1904}= 3.6$, $P<0.001$) and differed depending on whether bacteria or yeasts were considered (microbe x plant species $F_{41, 1904}= 3.8$, $P<0.001$).

Floral traits are associated with variation in microbial incidence and abundance

Plant species differed in microbial incidence in floral nectar (Figure 1) ranging from an average of 20% to 100% of sampled flowers containing bacteria. Floral traits were associated with some of this variation (Fig. 2A,B). Microbes were more frequently detected in nectar of plant species with an exposed nectary than those with short, mid or long corollas (Fig. 2; all flowers LRT=20.3, $P<0.001$), however, bacteria and fungi differed in patterns of occurrence (microbe x nectary location LRT=14.1, $P=0.003$). In other words, fungi were more frequently isolated from plant species with exposed nectaries and with long corollas (Fig. 2), while bacterial incidence was less variable among plant species with varying corolla length. Microbial incidence also differed among flowers depending on color (LRT=62.1, $P<0.001$), and bacteria and fungi differed in patterns of incidence among colors in some cases (microbe x color LRT 11.5, $P=0.009$). In particular, bacterial incidence was greater in red flowers than blue or light flowers,

whereas fungal abundance was not (Fig. 2). Microbial abundance also varied to some degree with flower traits, although bacteria and fungi differed in their responses. Specifically, fungi were most abundant in flowers with long corollas (Fig. 2 D microbe x nectary location $F_{3,1980}$ =8.5, P<0.001) compared to other flower morphologies, whereas bacterial abundance did not vary with nectary location. Microbial abundance did not vary significantly among flowers depending on floral color (color $F_{3,1980}$ =1.85, P=0.13).

Patterns of co-occurrence between bacteria and fungi

Among all individual flowers, bacterial and fungal incidence in nectar were positively correlated (Pearson's r=0.23; P=0.001). However, when flowers containing any microbial colonies were considered, bacterial and fungal incidence and abundance were negatively correlated (Incidence Pearson's r=-0.44; P<0.001; Abundance Pearson's r=-0.10; P<0.001). Notably, this relationship between bacterial and fungal abundance is variable among plant species, with most species displaying a negative or nonsignificant association, but some plant species showing a positive association (Supplementary Fig. 2).

Microbial associations with nectar volume and sugar

Standing nectar volume varied among plant species ($F_{41,1768}$=10.16, P<0.001), and was associated with microbial incidence and abundance, but in the opposite direction. Flowers containing no microbes had more nectar than flowers containing microbes (Bacterial presence t=-3.19, P=0.001; Fungal presence t=-2.12, P=0.03). In other words, nectar volume was lower when samples contained fungi or bacteria. However, microbial abundance in nectar was positively associated with nectar volume for both bacteria (t=4.01, P<0.001) and fungi (t=2.79, P=0.005). In addition, nectar sugar parameters varied significantly among plant species (amount $F_{14,251}$=18.94, P<0.001; concentration $F_{14,251}$=6.9, P<0.001; proportion monosaccharides $F_{14,251}$=8.9, P<0.001) and with nectar volume (amount t=21.1, P<0.001; concentration t=-1.46, P=0.14; proportion monosaccharides t=-4.5, P<0.001). Nectar sugar parameters were also significantly, albeit weakly, associated with microbial abundance in nectar. Sugar amount and concentration were negatively associated with fungal abundance in floral nectar (amount t=-2.3, P=0.02; concentration t=-1.9, P=0.05), whereas bacterial abundance was less strongly associated with variation in nectar sugars (amount t=-1.2, P=0.22; concentration t=-0.62, P=0.53). The

proportion of sugars comprised of monosaccharides was weakly positively associated with bacterial abundance in nectar (composition t=1.98, *P*=0.04) and fungal abundance was positively but not significantly associated with proportion of sugars comprised by monosaccharides (t=0.90; *P*=0.36).

Identity of culturable microbes

Bacteria from the genera *Acinetobacter*, *Bacillus*, *Pseudomonas,* and *Erwinia* were the most commonly identified from floral nectar (Supplementary Table 2). Yeasts from the genus *Metschnikowia* and *Candida* were the most commonly identified fungi (Supplementary Table 2). Filamentous fungi were not identified using MALDI.

Discussion

The patterns of bacterial and fungal occurrence in floral nectar observed here suggest that bacteria are less dispersal limited than are fungi among flowers. Greater dispersal ability of bacteria could be due to a few distinct factors. It may be that bacteria are able to use a great number of dispersal modes (e.g. wind, water, greater diversity of insects) compared to fungi, or that bacterial propagules are more easily dispersed or less susceptible to mortality during dispersal than fungal cells or spores. One alternative interpretation of the observed pattern is that fungi are equally able to disperse but less able to survive in floral nectar (immigration vs establishment (Cadotte 2006). Our survey data cannot directly address this possibility. All CFUs detected represent viable cells, and include species considered 'allochthonous' and 'autochthonous' to nectar (Brysch-Herzberg 2004)--species that are observed to attain high abundance and those that are only incidentally reported. Additional experiments would be required to compare if immigration vs establishment differed for multiple species of bacteria and fungi to assess which components of dispersal processes differ for bacteria and fungi. This could inform if bacteria and fungi differ in dispersal mode, viability during dispersal, or survival and establishment once reaching a new patch.

Two lines of evidence from our study support a primary role for floral visitors in dispersal of both culturable bacteria and fungi in floral nectar. First, the incidence of bacteria and fungi were positively correlated across individual flowers, suggesting shared vectors. Second, flowers

containing microbes had lower nectar volume compared to uncolonized flowers, consistent with a role of nectar consumers in dispersal of both bacteria and fungi. However, fungi are either more variable in establishment success, or less able to successfully disperse using these routes, resulting in their lower incidence overall (Fig 1). In particular, fungi (but not bacteria) are more likely to occur in and become abundant in flowers with long corollas (Fig 2). Notably, common nectar-inhabiting yeasts (*Metschnikowia reukaufii*, Supplementary Table 1) were frequently detected in flowers with long corollas, but not in exposed or short-corolla flowers. This pattern of fungal occurrence is consistent with a few non-exclusive hypotheses: 1) visitors of flowers with long corollas are primary vectors of fungi (yeasts) to flowers; and/or 2) fungi (yeasts) establish and grow most successfully in the microenvironments within long corollas, possibly explained by different nectar chemistry, reduced desiccation probability, or reduced UV exposure in plant species with long corollas (Plowright 1987). Our observations, together with previous work in bumble bees (Brysch-Herzberg 2004, Pozo et al. 2012b) and hummingbirds (Belisle et al. 2011, Lee et al. 2019) suggest the first hypothesis is likely, but do not rule out the second hypothesis. Specifically, flowers of plant species primarily pollinated by bumblebees or hummingbirds (e.g. *Delphinium* spp, *Castilleja* spp, and *Penstemon* spp, *Mimulus*(Diplacus)) contained the highest average densities of fungi (nearly all yeasts, Personal observation) (Figs 1-2, Supplementary Table 2). Exceptions to this pattern were also observed, however. For example, other studies have found the nectar of *Epilobium canum* to be abundantly colonized by yeasts (Morris et al. 2020), but fungi were infrequently detected in this species in this study, which we hypothesize is due to low levels of hummingbird visitation at this site during the sampling period. The nectar of the morning glory *Calystegia occidentalis* contained abundant yeast populations, but was frequently visited by solitary bees or flower beetles, which can also vector floral yeasts (Lachance et al. 2001). These data suggest that yeast may be more common and have stronger effects on plant-pollinator interactions in flowers with long corollas and in plant species that are frequently visited with effective yeast vectors. In contrast, bacterial are present across a greater range of plant species.

While dispersal is likely a key factor, other plant species suggest that environmental filtering can also shape nectar microbial communities. For example, nearly all flowers of *Toxicoscordion fremontii*, which produces very viscous nectar that is exposed on petal surfaces, contained

317 culturable microbes, but mostly at low densities. In *T. fremontii*, we hypothesize that abiotic
318 stressors (dessication, extreme osmotic conditions or UV), nectar characteristics (secondary
319 metabolites or other traits) could limit microbial survival, while infrequent microbial dispersal by
320 a limited visitor community (we only observed visitation by ants) may also limit immigration to
321 floral nectar.
322
323 In addition, correlations between bacterial and fungal incidence and abundance suggest roles of
324 both dispersal and competition in shaping microbial communities in nectar. Positive associations
325 between bacteria and fungi across flowers overall suggest shared dispersal vectors (Alvarez-
326 Pérez and Herrera 2013). Once flowers have been visited, however, bacterial and fungal
327 incidence and abundance were negatively associated at the level of individual flowers and often
328 within individual plant species (Supplementary Figure S2). Although we cannot rule out the
329 possibility that nectar conditions in some flowers may be more hospitable to yeasts compared to
330 bacteria, the evidence presented here is consistent with the hypothesis that competitive
331 interactions are important in nectar microbial communities, and suggest that priority effects
332 among nectar microbes may influence microbial diversity within individual flowers (Tucker and
333 Fukami 2014, Mittelbach et al. 2016, Tsuji and Fukami 2018). Our data do not directly inform
334 the processes governing changes in bacterial and fungal incidence or abundance through time
335 (Supplementary Figure S1), but they suggest that competitive interactions, together with
336 dispersal-mediated effects could contribute to priority effects across the flowering duration of
337 plant species and possibly through the field season. Investigating temporal patterns using more
338 detailed microbial community or strain-level analysis, combined with better resolution of
339 dispersal vector changes through time will better inform drivers of microbial community changes
340 through the flowering season.
341
342 Although the culture-dependent methods used in the current study may not be adequate to
343 characterize all microbes possibly found in this system, work on culture-independent
344 characterizations of multiple flowering plant species.(Álvarez-Pérez et al. 2012, Fridman et al.
345 2012, Vannette and Fukami 2017, Toju et al. 2018) suggest good correspondence between the
346 microbial taxa detected on the media types used here and with culture-independent approaches
347 (Morris et al. 2020). Although some colonies on YMA might be bacteria rather than fungi

(Dhami et al. 2018), this makes our comparisons conservative, given the possibility that some species might be detected on both media types and the lower detection threshold for colonies on YMA. In addition, our study reports a lower percentage of flower samples containing yeasts (fungi) than previous studies (Jimbo 1926, Herrera et al. 2009). A number of reasons may explain this disparity: is possible that cell density assessed microscopically may include unviable or unculturable cells, that culture methods have a higher detection threshold, or that our site has lower incidence than previously characterized sites. Our detection of bacteria yielded similar occurrence rates among flowers to previous culture-based descriptions of nectar bacteria (Álvarez-Pérez et al. 2012) despite different isolation media used.

Taken together, the data presented here suggest that fungi are more dispersal-limited than are bacteria among flowers. Previous work suggests that different processes may influence bacterial compared to fungal dispersal, in part due to their difference in size (Qian et al. 2012). Moreover, work from vector-borne plant pathogens suggests that a combination of microbe size, vector feeding mode and vector morphology are important in determining transmission (Mitchell 2004); similar processes may influence pollinator dispersal of bacteria vs fungi. Previous work in controlled metacommunities suggest that competition-colonization dynamics between bacterial strains affect coexistence and ecosystem productivity (Livingston et al. 2012). Such dispersal-competition tradeoffs may also structure species diversity for microbial assemblages on larger geographic scales, as has recently been demonstrated for ectomycorrhizal fungi (Smith et al. 2018). Our results extend this to interactions between bacteria and fungi. We suggest that further study of the consequences of variation in dispersal differences between bacterial and fungal taxa may inform the types and temporal dynamics of interactions between them, and consequences for host-microbe and multitrophic interactions in flowers.

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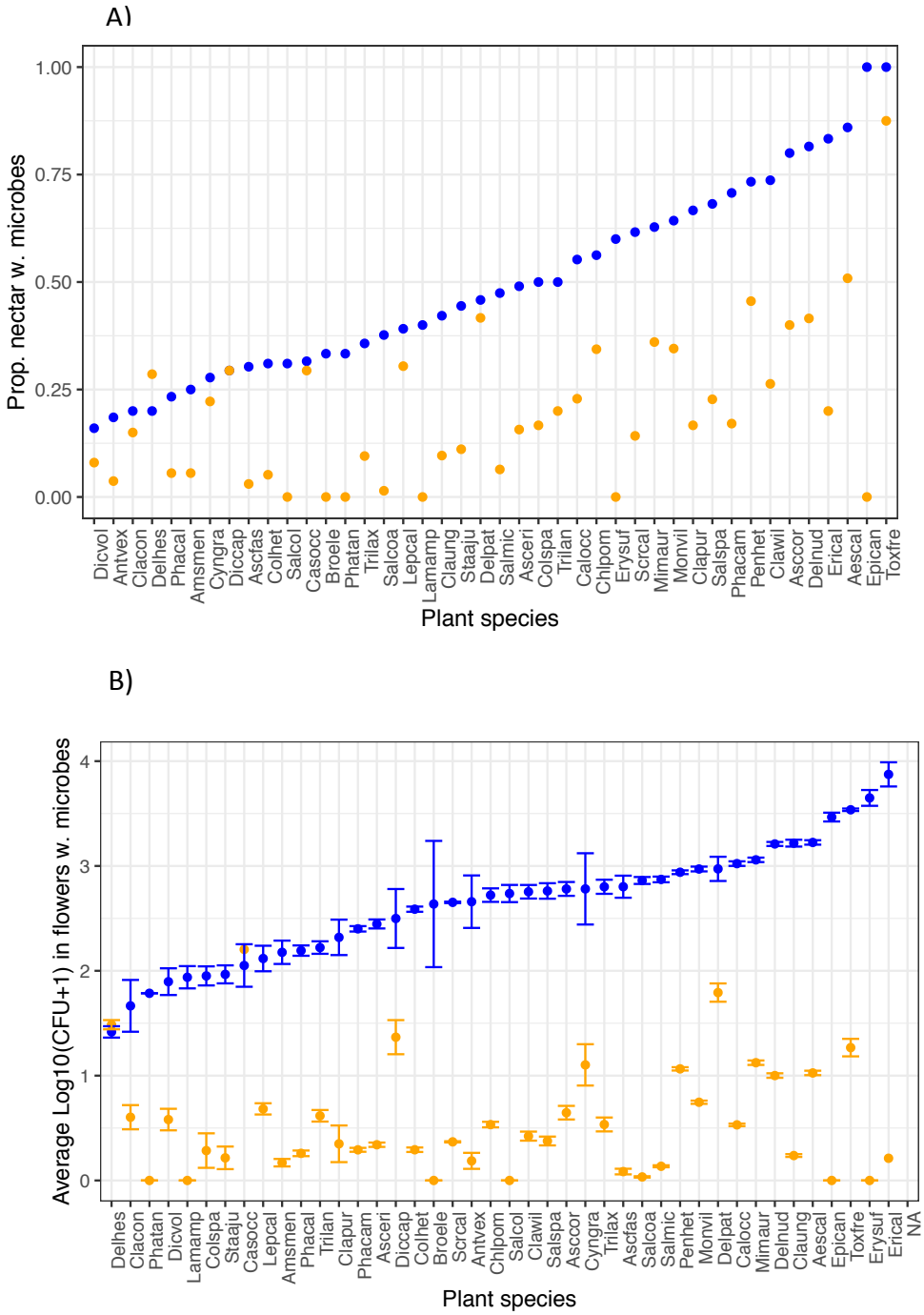

Figure 1. Plant species vary in a) the proportion of nectar samples containing colonies or b) the average abundance of CFUs on R2A media with antifungal compound ('bacteria', in blue) or colonies on YMA media with antibacterial ('fungi', in orange), ordered by increasing incidence of bacteria in samples. N=6-211 per species; median=23 nectar samples/species.

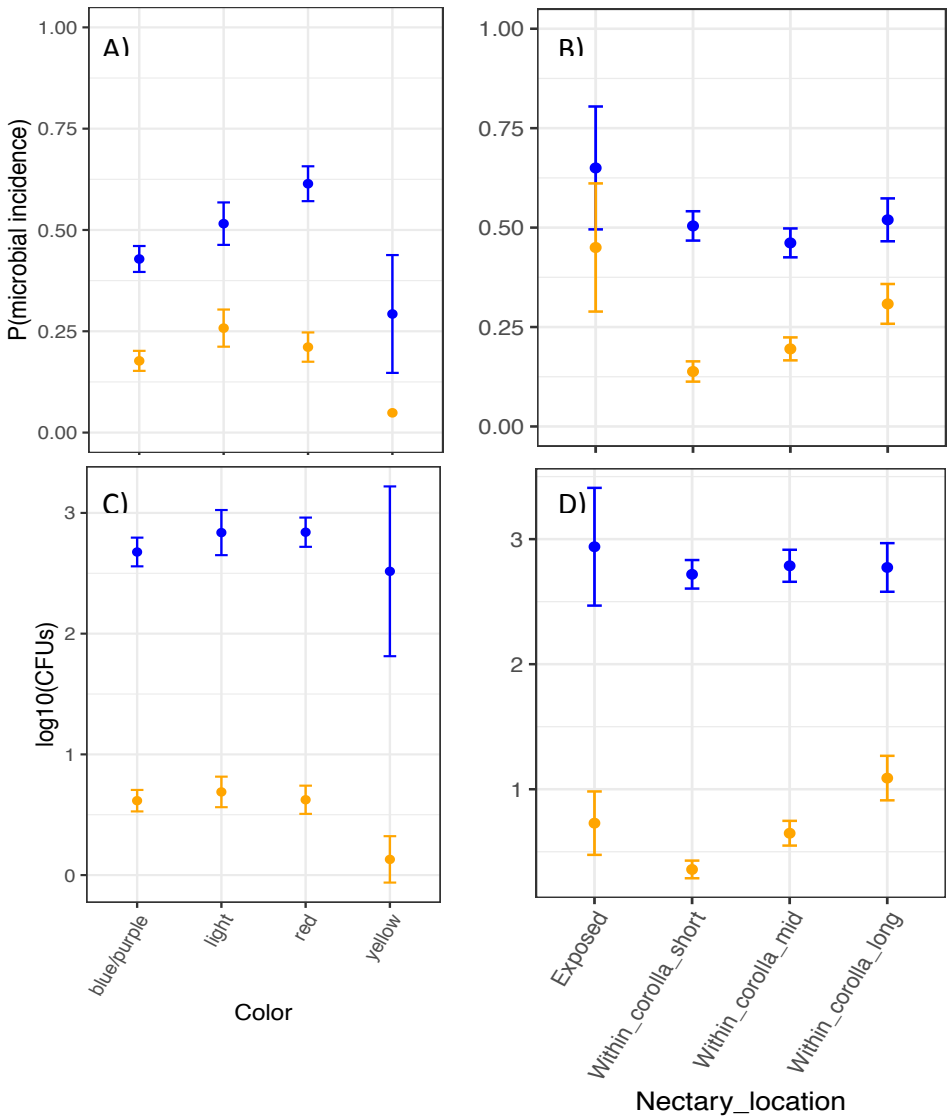

Figure 2. Microbial incidence and abundance in nectar varies with floral characteristics of host plant species including color (A,C) and location of the nectar (B,D). Average abundance was estimated using colony forming units counted on R2A and YMA media. Average values for bacteria are indicated in blue and fungi in orange. Microbial CFU abundance (C,D) are calculated using only those nectar samples containing detectable microbial growth, to discount variation in incidence. Points indicate value within a single nectar sample and include data from both sampling sites and years.

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521