Title: Fungi are more dispersal limited than bacteria among flowers

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Abstract:

Variation in dispersal ability among taxa affects community assembly and biodiversity maintenance within metacommunities. Although fungi and bacteria frequently coexist, their relative dispersal abilities are poorly understood. Nectar-inhabiting microbial communities affect plant reproduction and pollinator behavior, and are excellent models for studying dispersal of bacteria and fungi in a metacommunity framework. Here, we assay dispersal ability of common nectar bacteria and fungi in an insect-based dispersal experiment. We then compare these results to the incidence and abundance of culturable flower-inhabiting bacteria and fungi within naturally occurring flowers across two coflowering communities in California across two flowering seasons. Our microbial dispersal experiment demonstrates that bacteria disperse among habitat patches more readily than fungi via thrips. Across all flowers, bacterial and fungal incidence and abundance were positively correlated but bacteria were much more widespread, suggesting shared dispersal routes or habitat requirements but differences in dispersal and colonization frequency. The finding that bacteria are more common among flowers sampled here, in part due to superior insect-mediated dispersal, may have broad relevance for microbial life-history, community assembly of microbes and plant-pollinator interactions.

Introduction

Variation in dispersal ability among species can affect the composition and function of individual communities and biodiversity maintenance within metacommunities (Mouquet and Loreau 2003, Leibold et al. 2004). Dispersal limitation is well-documented for macro-organisms, and increasing evidence suggests that microbial taxa including fungi and bacteria can also be dispersal limited (Lindow and Andersen 1996, Peay et al. 2012, Albright and Martiny 2018, Svoboda et al. 2018). Despite the fact that bacteria and fungi often co-occur within communities, few studies have examined their relative dispersal abilities (but see Barberán et al. 2015). This gap in understanding is likely due to challenges in microbial detection, species delimitation using amplicon sequencing, and limited natural history knowledge of many microorganisms (Kraemer and Boynton 2016), particularly in a community context. As a result, comparisons in dispersal ability between coexisting bacteria and fungi are rare and limit the ability to predict the relative importance of dispersal for microbial community ecology and assembly dynamics. In addition, it is likely that processes underlying microbial community assembly and dynamics differ from those structuring communities of macroorganisms (Koskella et al. 2017). Characterizing dispersal differences of microbial taxa under realistic conditions is important to understanding how dispersal may shape microbial community assembly.

The microbial community in floral nectar—a sugar-rich reward that plants provide to pollinators-- is ecologically important and a tractable model system for comparing bacterial and fungal dispersal limitation (Chappell and Fukami 2018, Vannette 2020). The composition of bacteria and fungi in flowers can influence plant reproduction (Herrera et al. 2013, Schaeffer and Irwin 2014), either directly (Alexander and Antonovics 1988, Ngugi and Scherm 2006) or indirectly through effects on pollinator behavior and health (Vannette et al. 2013, Lenaerts et al. 2017, Sobhy et al. 2018). Bacteria and fungi often co-occur in nectar (Herrera et al. 2009, Fridman et al. 2012) where they can attain high abundance (Fridman et al. 2012, Tsuji and Fukami 2018). 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, Pozo et al.

2012, Alvarez-Pérez and Herrera 2013) and exhibit traits that may enhance fitness in nectar environments (Álvarez-Pérez et al. 2012, Pozo et al. 2012, Dhami et al. 2016a).

Despite strong initial environmental filtering, nectar-inhabiting microbial communities are ideal for examining dispersal limitation for a few reasons. First, communities in nectar undergo primary succession (Chappell and Fukami 2018). Few culturable microbes are found in nectar of newly opened flowers that have not received visitation by animals (Brysch-Herzberg 2004b, Vannette and Fukami 2017, von Arx et al. 2019, Morris et al. 2020), 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 culturable (Morris et al. 2020)— shotgun sequencing of nectar microbial communities reveal qualitatively similar taxonomic composition to culture-based analyses. Third, bacteria and fungi in nectar are thought to depend on phoresy via flower visitors (Brysch-Herzberg 2004b, Vannette and Fukami 2017, Morris et al. 2020). Fourth, plant species differ in flower morphologies associated with variation in nectar accessibility to wind and animal pollinators.

In addition, the assembly processes shaping nectar microbial communities are thought to depend on differential dispersal limitation. Laboratory experiments and field observations suggest that inhibitory priority effects (interactions in which species arrival order determines their outcome) influence community structure in floral nectar (Tucker and Fukami 2014, Toju et al. 2018) via differential dispersal of species to flowers (Mittelbach et al. 2016). 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 and co-occurrence in nectar would shed light on the mechanisms underlying their community assembly, inform the competitive environments experienced by bacteria and fungi in nectar, and have implications for microbial effects on interactions between plants and floral visitors.

109 Here, we test the hypothesis that nectar-inhabiting bacteria and fungi differ in dispersal
110 and occupancy among individual flowers in two coflowering communities. We assess
111 realized dispersal—which integrates the components of emigration (leaving a habitat),
112 survival during transport, and establishment in a new habitat (flower)—which ultimately
113 determines community membership (Baguette et al. 2012). First, we examined microbial
114 dispersal ability empirically and second we assessed bacterial and fungal occupancy
115 (incidence) and abundance in individual nectar samples of more than 1800 flowers,
116 spanning 43 species of plants at two sites to examine 1) do bacteria or fungi differ in
117 dispersal ability by a common flower-visiting insect; 2) are patterns of bacterial and
118 fungal incidence or abundance in nectar similar across a plant community; 3) does
119 microbial presence or abundance differ among plant species or with floral accessibility
120 (flower morphology); 4) does season, year or site predict patterns of bacterial or fungal
121 incidence and abundance?
122
123
124 **Materials and methods**
125 *Insect-based microbial dispersal assay*
126 To empirically test whether nectar microbes differ in their dispersal ability, we developed
127 an assay of microbial dispersal resulting from the feeding activity of western flower
128 thrips (*Frankliniella occidentalis*). The assay consisted of 5 sterilized adult female thrips
129 freely foraging within a 96-well plate in which 1/3 of wells contained a microbial
130 suspension (200uL 10,000 cells/uL) in sweetened tryptic soy broth (TSB, adding 15%
131 sucrose and 15% fructose) and the remaining 2/3 of wells were filled with sterile TSB
132 (200uL). Thrips were sourced from a colony held by Diane Ullman (see Supplementary
133 Figure, Methods S1 for assay methods details). Microbial isolates from field sampling in
134 this study (see below and Supplementary Table S1), and other nectar-associated
135 microbe collections were used (6 bacteria, 5 fungi). After thrips foraged for 24 hours at
136 30°C under 16L:8D light, thrips were killed by adding 320uL of ethyl acetate to the plate
137 bottom and the media was incubated for 5 additional days. Control replicates with thrips
138 and no added microbes were included in all trials to ensure adequate sterilization and
139 trial consistency. Following incubation, we assessed microbial occupancy of initially

140 sterile wells by calculating the deviation of optical density (OD 600nm) from a blank
141 control plate (no thrips, no microbes) with an occupancy threshold equal to the mean
142 OD of control wells +6 standard deviations.
143
144 *Coflowering community sampling for bacteria and fungi*
145 To assess patterns of microbial incidence and abundance, we sampled standing crop
146 floral nectar during 2016 and 2017 at two sites in northern California: Stebbins Cold
147 Canyon Reserve (38.49806°, -122.09931° 140m, hereafter Stebbins) in Winters, CA
148 and at flowering plots maintained at the Laidlaw Honey Bee Facility (38.53653°, -
149 121.78864°, 19m, hereafter Bee Biology) in Davis, CA, which hosts an active apiary.
150 The sites are approximately 30 kilometers apart, and differ in pollinator species
151 composition and anthropogenic influence, but share a subset of plant species.
152
153 Between late February and early July, plant communities were sampled every two to
154 four weeks. We collected approximately 10 flowers from abundant plant species. In
155 some cases, collections were limited by floral availability and reserve collection
156 restrictions to protect plant populations. When possible, flowers were sampled from
157 multiple individual plants and sub-populations or plots (Supplementary Table S2). Care
158 was taken to sample flowers that had been open more than one day when possible, to
159 allow the opportunity for floral visitation and microbial immigration to flowers. Individual
160 inflorescences were collected, placed upright in humidified boxes and kept cool until
161 nectar extraction and plating, no more than 5 hours later. In the lab, flowers were
162 destructively sampled. Nectar was collected using 10 µl microcapillary tubes, and its
163 volume was quantified. Nectar was diluted in 30 µl of sterile water (D0), then diluted 10
164 and 100 fold (D1 and D2 respectively) in sterile phosphate-buffered saline (Dulbecco's,
165 pH 7.1-7.5). To assess fungal and bacterial abundance, 50 µl of the 10 fold dilution
166 (containing 5 µl of D0) and 50 µl of the 100 fold dilution (containing 0.5 µl of D0) were
167 plated on yeast media agar (YMA) containing the antibacterial chloramphenicol and
168 Reasoner's agar containing the antifungal cycloheximide (R2A Oxoid formula with 16%
169 sucrose), respectively. All samples were plated the day of collection. For convenience,
170 we refer to the total number of colonies on YMA as "fungi" and colonies on R2A as

171 “bacteria” throughout the manuscript although some colonies on each media type may
172 be comprised by microbes resistant to the antimicrobial compounds used here (e.g.
173 bacteria resistant to chloramphenicol (Dhami et al. 2018)). Based on dilution curves, we
174 were able to detect CFUs on plates at approximately 10^1 live cells in the original nectar
175 sample for YMA and 10^2 cells for R2A (Supplementary methods S2 and Figure S2).
176
177 Negative controls were plated for each sampling bout to detect potential
178 contamination and samples were discarded if contamination was detected (detected for
179 1 collection date; these samples were removed from the analysis). Agar plates were
180 incubated at 28°C and colony-forming units (CFUs) were counted after 48-72 hours.
181 The total number of CFUs and CFU density for each nectar sample was calculated
182 based on dilutions and original sample volume. Over the course of the study, 1,825
183 nectar samples were collected and plated on two media types. In 2016, colonies were
184 picked haphazardly from plates collected over the entire season including all sites and
185 plant species and frozen in glycerol.
186
187 *Nectar accessibility: flower corolla length*
188 Microbial dispersal to nectar is likely affected by the location and accessibility of floral
189 nectar within the flower. Specifically, nectar that is not enclosed by flower tissue may be
190 more easily accessed via wind dispersal and may also be accessible to a greater
191 diversity of insect visitors. On the other hand, nectar protected by flower tissues
192 comprising the perianth (non-reproductive structures of the flower) may be less
193 accessible via wind dispersal and contacted only when flowers are visited by long-
194 tongued birds or insects (Kingston and Mc Quillan 2000, Lara and Ornelas 2001). We
195 examined if bacterial or fungal incidence or abundance depended on nectar location
196 within the flower (flower corolla length). Nectar within flowers of each species were
197 classified as “exposed” (no corolla/exposed nectary), or contained within “short” (1-5
198 mm), “mid” (5-15 mm), or “long” (15+ mm) perianth, based on corolla tube length
199 (Supplementary table S2).
200
201 *Microbial identification: culture-based and culture-independent analyses*

202 A subset of microbial strains from glycerol stocks were identified using MALDI-TOF and
203 spectra were compared to Bruker Bacteria and Eukaryote libraries and a custom in-
204 house database curated from previously identified microbial isolates from nectar
205 (Supplementary methods S3). In addition, we used a culture-independent sequencing
206 approach to characterize diversity in full-length 16S and ITS sequences from 25 pooled
207 nectar samples (239 flowers in each sample), representing multiple sampling dates at
208 each site from 2016. Pooled nectar samples were extracted using the Nucleospin
209 Microbial DNA kit (Macherey-Nagel, Bethlehem, PA, USA) and submitted for full-length
210 PacBio16S and ITS sequencing performed at Dalhousie IMR. Recovered sequences
211 were processed using dada2 pipeline for full-length rRNA genes (Callahan et al. 2016,
212 Callahan et al. 2019) for recovery of actual sequence variants (ASVs). Briefly, primer
213 sequences were removed, and resulting sequences were quality filtered, error-corrected
214 and denoised followed by taxonomy assignment using SILVA v. 138 training set (Quast
215 et al. 2012) for bacteria and UNITE 4/2/2020 release for fungi (Kõljalg et al. 2005)
216 followed by chimera detection and removal (See Dryad https://doi.org/10.25338/B8403V
217 for bioinformatics scripts). ASVs annotated as chloroplast or plant, and those that were
218 also obtained from extraction blanks were removed.
219
220 *Statistical analysis*
221 To examine if microbes differed in dispersal ability in our experimental setup, the
222 proportion of wells colonized by a microbe within a plate (calculated using OD as
223 described above; plates as replicates) was analyzed using a binomial GLMM (package
224 lme4) to compare bacterial and fungal dispersal plates with strain as a random effect. A
225 t-test was also used to compare bacteria and fungi by the mean proportion of wells
226 colonized by each strain across 8 replicate plates.
227
228 To examine the relationship between bacterial and fungal incidence and abundance in
229 individual nectar samples, we used Pearson correlations. In addition, we report directly
230 calculated conditional probabilities and a χ^2 test to test for non-independence of
231 bacterial and fungal incidence amongst flowers.
232

233 To compare plant species differed in bacterial or fungal presence or abundance, we
234 used logistic regression and linear regression with plant species as a predictor. To
235 compare if nectar accessibility quantified using corolla length category was associated
236 with bacterial or fungal presence or abundance, we used logistic regression and linear
237 regression with nectary location (exposed, short corolla, mid corolla or long corolla) as a
238 predictor.
239
240 To determine if bacteria and fungi differ in incidence in floral nectar with Julian date,
241 between sites, or between years, we used a logistic regression implemented using
242 'glm', using bacteria or fungal presence as a response and site, year, and plant species,
243 and their two-way interactions, as predictors. We compared among: models containing
244 all interactions, models with non-significant interactions removed, and models with no
245 interactions using AIC, and from the best-fit model, significance of each term was
246 assessed using likelihood ratio tests. To determine if bacteria or fungi differ in
247 abundance in floral nectar across years or sites, or with Julian date, we used a linear
248 regression implemented using 'lm', with $\log_{10}$(CFUs+1) as a response and site, Julian
249 date, and year as predictors. Model selection was performed on bacteria and fungi
250 separately using AIC as described above, and significance was assessed using
251 likelihood ratio tests.
252
253 We assessed the frequency with which microbial genera were detected using MALDI-
254 TOF among flower morphologies, or across the season (early, mid, or late, see
255 supplement for details) using a χ^2 test for contingency tables. Microbial composition
256 from PacBio sequencing is presented to compare taxonomic composition with MALDI-
257 TOF data but due to limited numbers of samples successfully sequenced, no formal
258 analysis was performed. All analyses were conducted using RStudio and R v. 3.6.3
259 (Johnson et al. 2009).
260
261 **Results**
262 *Insect-based dispersal assay*

263 Fungi were less able to disperse among wells than bacteria in the thrips dispersal assay
264 (Figure 1). Using strain-level averages to compare dispersal abilities between
265 kingdoms, bacteria colonized 31% more wells than did fungi over 24 hours of thrips
266 activity (t-test, strains as replicates, $t = 4.31$, $p=0.002$). Results from the GLMM confirm
267 this difference between fungi and bacteria average dispersal ability (z-value =-4.66,
268 $p<0.001$). Control treatments had detectable, but low levels of contamination (higher
269 than two fungal strains) despite exhaustive measures to sterilize thrips.
270
271 *Patterns of bacterial and fungal incidence and co-occurrence in floral nectar*
272 Floral nectar samples from the field contained colonies on R2A media (hereafter
273 'bacteria') in 49% of samples, whereas colonies on YMA (hereafter, 'fungi') were
274 detected in only 20% of samples (microbe LRT=21.07, $p<0.001$, Figure 2). Fungi and
275 bacteria co-occur in flowers nearly 1.5 times as frequently as would be expected by
276 chance given their individual incidence across the landscape (14% of flowers contain
277 both, compared to 9% of flowers estimated joint probability if independent, $\chi^2=94.37$,
278 $p<0.001$). Along with the difference between bacterial (49%) and fungal (20%) incidence
279 across the landscape, this elevated frequency of co-occurrence results in a discrepancy
280 between the likelihoods that fungi or bacteria find themselves co-occupying a flower
281 (conditional probabilities of co-occupancy for each microbe). Fungi (most often yeasts)
282 are most often found in flowers alongside bacteria (P(bacteria present | fungi present) =
283 69%). On the other hand, bacteria only occasionally co-occupy a flower with fungi
284 (P(fungi present | bacteria present) = 29%).
285
286 Moreover, fungi and bacteria demonstrate a positive correlation in CFU abundance
287 across flowers (Pearson correlation, $r = 0.27$, $p<0.001$), which persists even when
288 investigating only flowers that contain both bacteria and fungi (Pearson correlation,
289 $r=0.24$, $p<0.001$). Within plant species, fungi and bacterial incidence and abundance are
290 most often positively correlated, but only significantly in four species (Supplementary
291 Figure S3).
292
293 *Nectar location associated with variation in microbial incidence and abundance*

294 Plant species differed in microbial incidence (bacteria LRT=306, $p<0.001$; fungi
295 LRT=271.09, $p<0.001$) and abundance (bacterial CFUs $F_{41,1816}=9.3$, $p<0.001$; fungal
296 CFUs $F_{41,1816}=7.3$, $p<0.001$) in floral nectar (Figure 3) ranging from an average of 20%
297 to 100% of sampled flowers containing bacteria. Nectar location within a flower was
298 associated with some of this variation (Figure 4). Both bacteria and fungi were more
299 frequently detected in nectar of plant species with an exposed nectary than those with
300 short, mid or long corollas (Figure 4; bacteria LRT=8.1, $p=0.04$; fungi LRT=52.9,
301 $p<0.001$). Microbial abundance also varied with flower traits, but bacteria and fungi
302 differed in their responses. Bacteria were more abundant in flowers with exposed nectar
303 ($F_{3,1813}=4.65$, $p=0.003$) whereas fungal CFUs were most abundant when nectar is
304 contained within a long corolla (Figure 4; $F_{3,1810}=29.1$, $p<0.001$) compared to other
305 flower morphologies.
306
307 *Sources of variation in bacterial and fungal incidence and abundance*
308 Microbial incidence differed between geographic sites and years, and changed through
309 the season (Julian date) (Supplementary Tables S3, Figure S4). Bacteria and fungi
310 differed in which site each was more detectable and abundant, with bacteria more
311 frequently detected and abundant at the Bee Biology site (Table S3) and fungi more
312 frequently detected and abundant at the natural reserve site (Table S3). The probability
313 of detection of both bacteria and fungi changed across the season—most often
314 increasing with Julian date—and among years and sites relatively similarly (Figure S4),
315 whereas patterns of fungal and bacterial abundance tended to differ in response to
316 seasons and years.
317
318 *Microbial identification and genus-specific patterns*
319 Bacteria from the genus *Acinetobacter* were the most common bacterial genera
320 detected based on MALDI and PacBio sequencing, (Supplementary Table S4, Figure
321 S5). Yeasts from the genera *Metschnikowia* and *Candida* were the most commonly
322 identified fungi (Supplementary Table S4, Supplementary Figure S5). Filamentous fungi
323 could not be identified using MALDI, but *Penicillium*, *Cladosporium*, and *Epicoccum*
324 were detected using PacBio sequencing of the ITS region.

Microbial genera differed in their patterns of incidence among flowers of different morphologies. The bacterial genus *Acinetobacter* was significantly overrepresented in flowers with short corollas (Supplementary Figure S6), whereas *Pseudomonas* and the yeast *Metschnikowia* were significantly overrepresented in flowers with long corollas. Seasonal patterns were also observed: *Lactobacillus* and *Pseudomonas* were significantly overrepresented in the early season, whereas *Acinetobacter* and *Metschnikowia* were significantly underrepresented in flowers during the early season (Supplementary Figure S7).

Discussion

In our survey, bacteria were more commonly detected in floral nectar than were fungi, and greater dispersal ability of bacteria in the experimental dispersal assay provides support for the hypothesis that differences in dispersal ability shape bacterial and fungal occupancy of coflowering communities sampled in northern California. Our use of culture-based detection is conservative in detecting bacteria (Supplementary methods S2 and Supplementary Figure S2), and the taxa detected in our sequencing efforts matched well to those detected in culture analyses, suggesting that our sampling represented well the majority of microbial community members. Our results reveal that bacteria and fungi differ in dispersal ability between flowers but frequently coexist across this and many other metacommunities. Fungal dispersal limitation may be compensated for by previously characterized differences in life histories including attraction of insect vectors by fungi or competitive exclusion of bacteria through priority effects.

Nectar-inhabiting bacteria and fungi are both likely to rely on frequent dispersal (Madden et al. 2018, Vannette 2020) because individual flowers typically persist a short time (hours to days) (Primack 1985). Previous studies and our own observations suggest multiple adaptations in both bacteria and yeasts for enhanced dispersal. Bacteria from the dominant genera found in nectar (e.g. *Acinetobacter*, *Rosenbergiella*) form sticky biofilms (Irie and Parsek 2008, Jung and Park 2015, Álvarez-Pérez et al.

356 2019), which could enhance adhesion to insect vectors. In addition, the yeasts common
357 to nectar exhibit cell morphology that may improve adhesion to insect or bird
358 mouthparts (e.g. 'airplane' cells in the yeast *Metschnikowia gruessi* (Brysch-Herzberg
359 2004b)). Bacteria and fungi, including yeasts, could produce stress-tolerant spores that
360 enhance desiccation resistance (Francisco et al. 2019) and survival during transport
361 between flowers. Microbial volatile compounds (Vannette et al. 2013, Rering et al. 2018)
362 also affect vector attraction to flowers and visit duration (reviewed in Crowley-Gall et al.
363 2020). The greater dispersal ability of bacteria observed here could be due to enhanced
364 vector attraction, ease of attachment to insect vectors, persistence in association with,
365 survival on, or localization within hosts (Perilla-Henao and Casteel 2016), or more
366 efficient colonization success of nectar, but further study would be required to
367 disentangle the specific mechanisms that underlie bacterial dispersal ability.
368
369 Bacterial numerical dominance and superior dispersal ability could predict bacterial
370 exclusion of fungi in nectar over ecological time via mass effects (Leibold et al. 2004),
371 so it may be notable that fungi persist in the nectar environment. Coexistence between
372 bacteria and fungi could be explained by a few factors. First, different modes of
373 dispersal could favor different microbial species. For example, some yeasts may
374 enhance attraction, specialize in attachment, or engage in persistent associations with
375 particular flower visitors, as has been documented for bumble bees and *Metschnikowia*
376 yeasts (Brysch-Herzberg 2004, Herrera et al. 2013, Jacquemyn et al. 2020). When
377 those flower visitor species are abundant, yeast dispersal and abundance in the floral
378 nectar metacommunity is predicted to increase. Similarly, spore-forming fungi may rely
379 on wind dispersal, although the incidence and abundance of filamentous fungi in floral
380 nectar was very low in our survey. Second, plant species may differ in suitability for
381 colonization by yeasts compared to bacteria (Figures 3 & 4) or differ in flower conditions
382 that affect yeast competitive ability. Floral nectar in some species contains antimicrobial
383 defenses (González-Teuber and Heil 2009) including antifungal and antibacterial
384 enzymes (Thornburg et al. 2003, Carter and Thornburg 2004, Roy et al. 2017, Schmitt
385 et al. 2018), some of which could differentially reduce bacterial growth compared to
386 yeast growth. Here, flowers with long corollas which host abundant yeast populations

387 not reflected in bacterial populations. However, whether visitation by long-tongued
388 pollinator taxa (Brysch-Herzberg 2004a, Pozo et al. 2012), or floral traits including
389 reduced desiccation probability and reduced UV exposure (Plowright 1987) could
390 enhance yeast colonization or growth in flowers with long corollas is unclear. Third,
391 interference competition by fungi, including production of antimicrobial compounds,
392 nutrient scavenging ability (Dhami et al. 2016), or environmental modification (Tucker
393 and Fukami 2014, Vannette and Fukami 2014) could lead to suppression of bacterial
394 populations in longer-lived flowers (but see discussion below).
395
396 Our data also reveal genus-specific patterns of plant colonization and seasonal change
397 for a few common nectar-inhabiting microbes that may shed light on their dispersal
398 ecology. Yeasts from the genus *Metschnikowia* were most frequently isolated from
399 flowers with longer corollas (Supplementary Figure S6), consistent with previous reports
400 of reliance on large-bodied bees and hummingbirds for among-flower dispersal (Herrera
401 et al. 2010, Vannette and Fukami 2017, Morris et al. 2020). In contrast, bacteria from
402 the genus *Acinetobacter* were more frequently isolated from flowers with shorter
403 corollas, indicating either different dispersal vectors or the ability to successfully
404 colonize flowers with different morphologies compared to *Metschnikowia*. For both of
405 these nectar-inhabiting genera, incidence increased over the season. We note that
406 many relevant ecological factors change in tandem seasonally: temperature increases,
407 the number of flower-visiting insects increases and change in species composition, and
408 floral density also changes across the flowering period sampled here. Previous work
409 has shown that the flower visitor community is associated with microbial community
410 composition and network properties in a coflowering community (Zemenick et al. 2021)
411 but whether turnover in flower visitor species or other seasonal changes are the most
412 important drivers of microbial composition over time is unclear.
413
414 Distinct patterns of bacterial and fungal presence and abundance in floral nectar
415 documented here have a few specific implications for the ecology and evolution of the
416 floral microbiome. For more prevalent nectar bacteria, inter-kingdom competition may
417 be less important in the evolution of bacterial traits and life-history than for fungi, which

418 are frequently exposed to competition with bacteria (Figure 2). Some models suggest
419 that conditions of frequent yeast-bacterial interactions favor the evolution of antibacterial
420 production by yeasts (Zhou et al. 2018). Nectar yeast have been hypothesized to
421 produce metabolites that reduce bacterial growth (Álvarez-Pérez et al. 2019) and nectar
422 yeast genomes contain scaffolds annotated as containing genes with potential
423 antimicrobial function, including polyketide synthase-like and non-ribosomal peptide
424 synthetase cluster-like (Nordberg et al. 2014), but experimental work is lacking. In
425 contrast, experimental and genomic evidence suggests that *Metschnikowia reukaufii*
426 benefits from rapid nutrient assimilation (Dhami et al. 2016) and population growth
427 (Pozo et al. 2012), suggesting maximizing propagule abundance is an important
428 strategy for nectar microbial populations (Hibbing et al. 2010). Moreover, our data do
429 not reveal strong negative correlations between fungi and bacterial abundance in
430 nectar, either among or within species (Supplementary Figure S3), suggesting that
431 interference competition may not be strong in our focal populations. Instead, we noted
432 positive correlations between fungal and bacterial abundance at the flower level, which
433 could indicate shared dispersal vectors, similar habitat requirements for culturable
434 nectar microbes, or the possibility of facilitation which could be promoted by bacterial
435 enhancement of nutrients in nectar from pollen or other plant sources (Christensen et al.
436 2021).
437
438 Taken together, the data presented here demonstrate that common nectar-inhabiting
439 fungi are more dispersal limited than are bacteria and less frequently detected among
440 flowers. Previous work has shown that dispersal can be important in structuring
441 bacterial community assembly (Albright and Martiny 2018), and generate the possibility
442 of historically contingent competitive outcomes in microbial communities (Fukami 2015,
443 Svoboda et al. 2018). By demonstrating clear differences in dispersal ability, our results
444 extend this to interactions between bacteria and fungi. We suggest that further study of
445 the consequences of variation in dispersal differences between bacterial and fungal
446 taxa may inform the types and temporal dynamics of interactions between them, and
447 consequences for host-microbe and multitrophic interactions in flowers.
448

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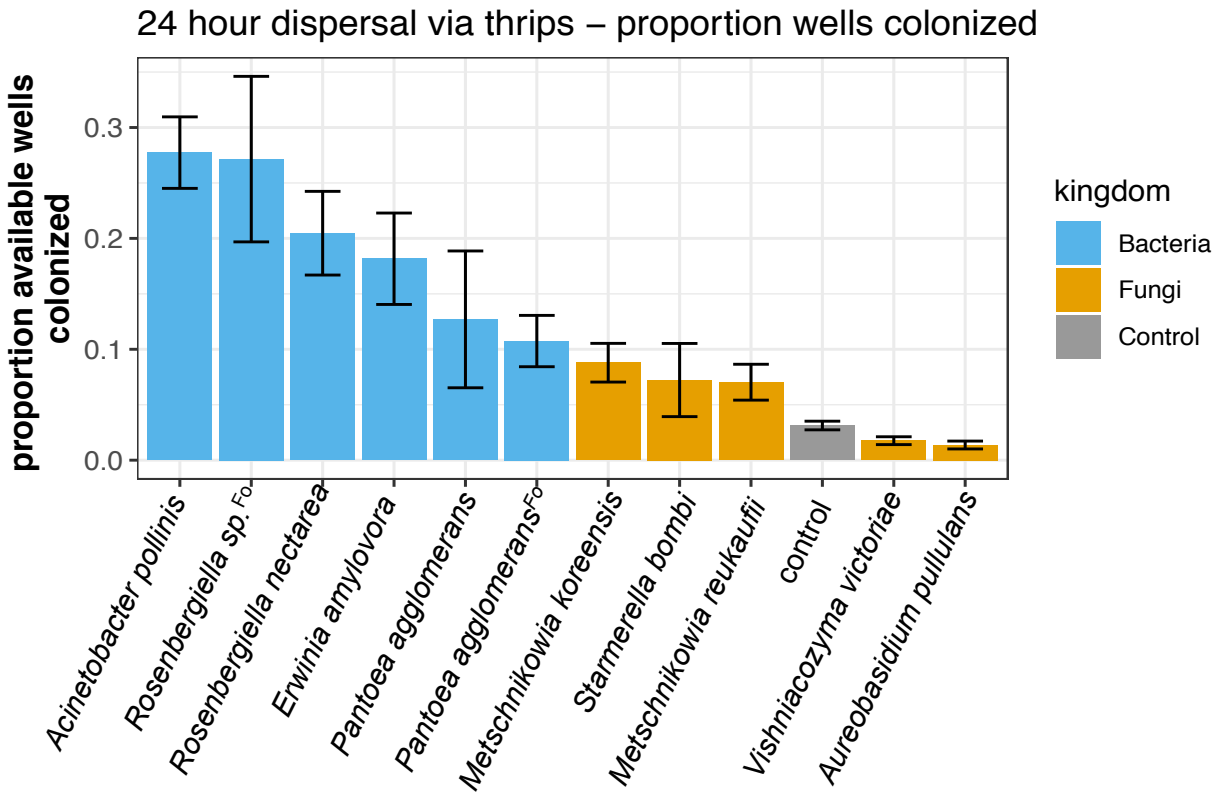

Figure 1 – Western flower thrips (*Frankliniella occidentalis*) disperse bacteria between artificial nectar habitats more readily than fungi. Each bar shows the relative success of a different isolate of bacteria or fungus in colonizing wells on a 96-well plate through the activity of sterilized thrips (N=8-12 plates per isolate). Microbes with superscript 'Fo' were isolated from *Frankliniella occidentalis*. The control bar represents trials in which all wells began sterile and only thrips were added, representing contamination levels from thrips only. Bar heights report mean values (+/- 1 S.E.) for the proportion of available well's colonized after 24 hours of thrips activity and a subsequent 6 days of culturing in an incubator.

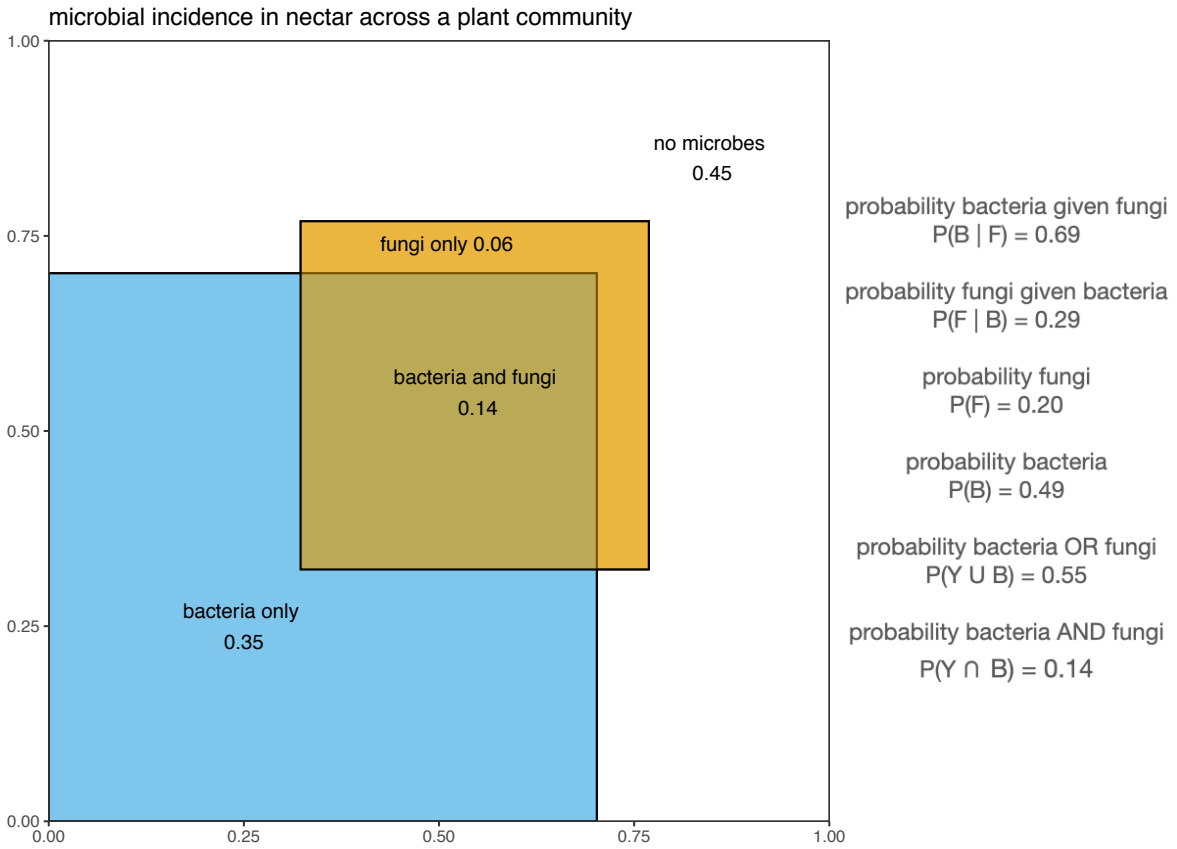

Figure 2 – Bacterial and fungal incidence in nectar in the two flowering plant communities sampled. The figure shows the relative incidence of bacteria and fungi in individual nectar according to shaded areas and their proportions of flower occupancy. Bacteria occur in more flowers than fungi across the landscape. The two kingdoms co-occur within nectar more often than would be predicted by chance. Fungi rarely occur in the absence of bacteria, but bacteria frequently occur in the absence of fungi.

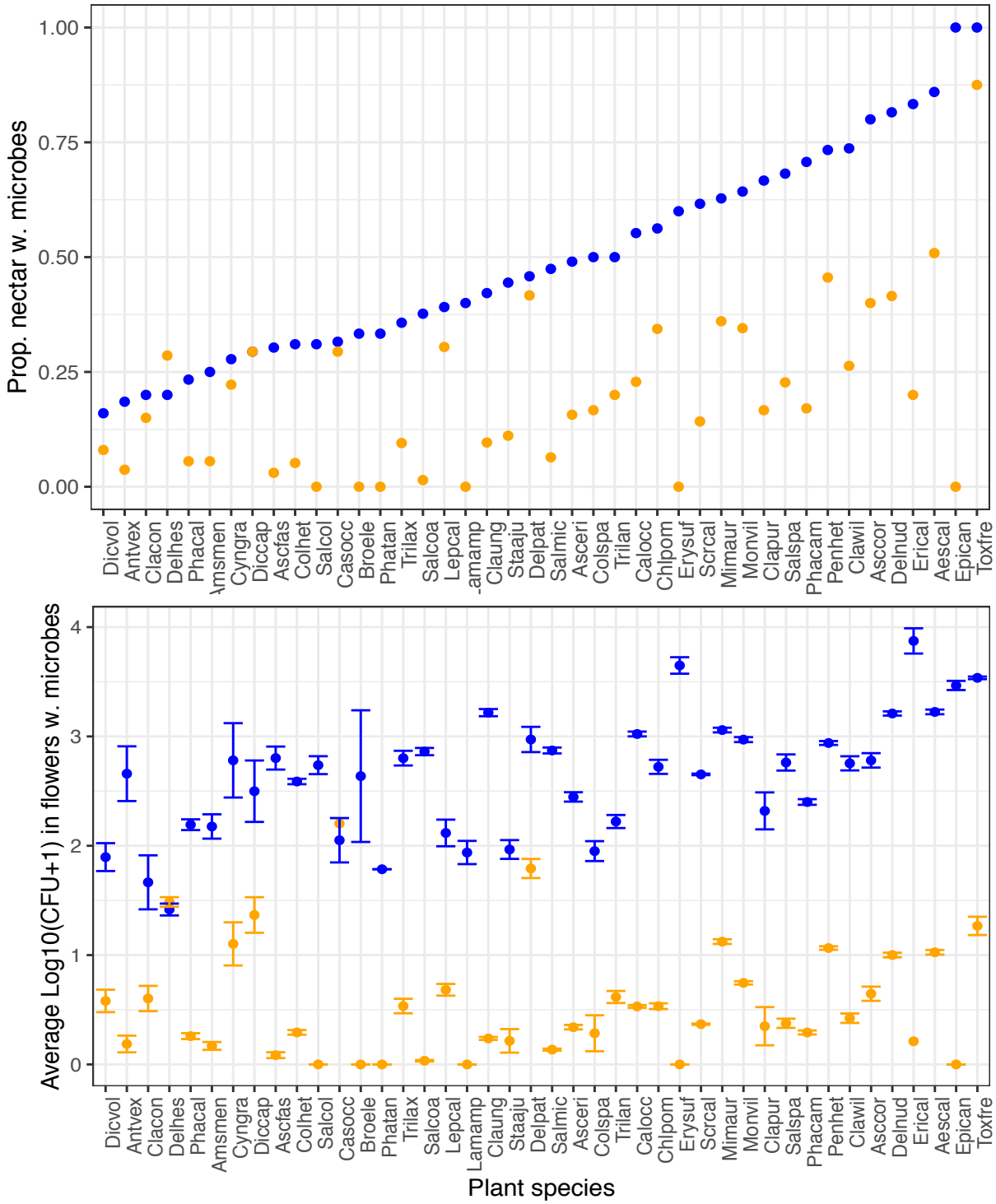

Figure 3 –Plant species vary in A) the proportion of total nectar samples containing colonies or B) the average calculated abundance of CFUs in microbial-colonized nectar on R2A media with antifungal compound ('bacteria', blue) or colonies on YMA media with antibacterial ('fungi', orange), ordered by increasing incidence of bacteria in samples. N=6-211 per species; median=23 nectar samples/species.

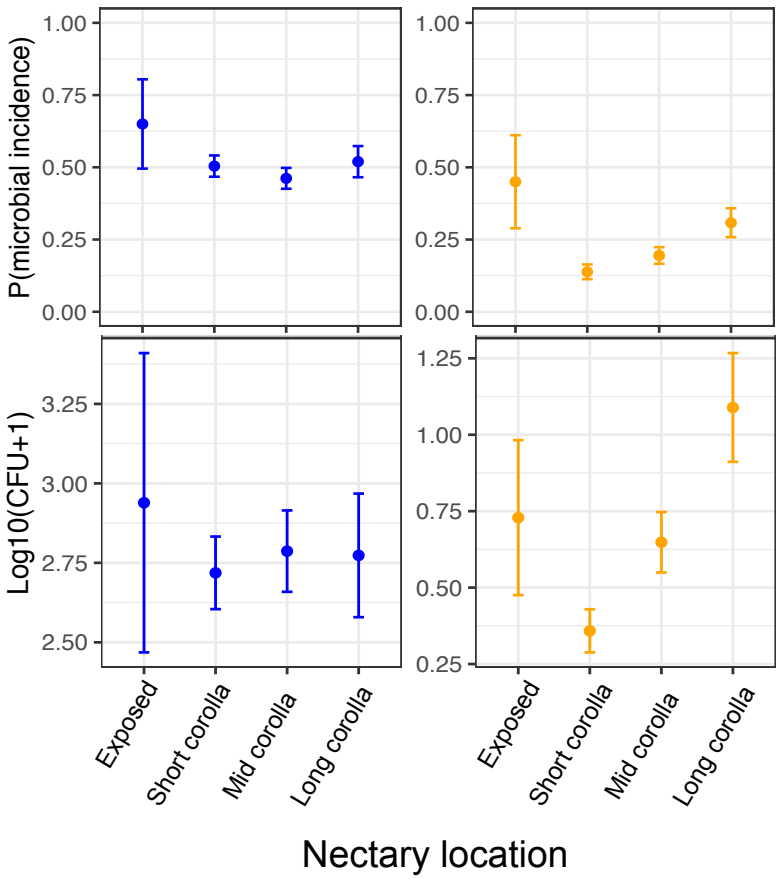

Figure 4 – Microbial incidence and abundance in nectar varies location of the nectar (corolla length). Average microbial abundance in flowers was calculated using colony forming units counted on R2A (blue, bacteria) and YMA (fungi, orange) media and adjusted based on nectar volume. Microbial CFU abundance calculated using only those nectar samples containing detectable microbial growth. Note difference between y-axes between bacteria and fungi in microbial abundance.