FEMS Microbiology Ecology, 97, 2021, fiab150

https://doi.org/10.1093/femsec/fiab150
Advance Access Publication Date: 17 November 2021
Research Article

RESEARCH ARTICLE

Culturable bacteria are more common than fungi in floral nectar and are more easily dispersed by thrips, a ubiquitous flower visitor

Rachel L. Vannette*,†, Marshall S. McMunn‡, Griffin W. Hall, Tobias G. Mueller§, Ivan Munkres¶ and Douglas Perry#

Department of Entomology and Nematology, University of California Davis, Davis, CA 95616, USA

*Corresponding author: Department of Entomology and Nematology, University of California Davis, Davis, CA 95616, USA. Tel: +1-530-752-3379; E-mail: rlvannette@ucdavis.edu

One sentence summary: Bacteria outnumber fungi in northern California flowers and are more easily dispersed by thrips, common flower-visiting insects.

Editor: Julie Olson

†Rachel L. Vannette, https://orcid.org/0000-0002-0447-3468
‡Marshall S. McMunn, https://orcid.org/0000-0002-0585-8722
§Tobias G. Mueller, https://orcid.org/0000-0002-6127-3091
¶Ivan Munkres, https://orcid.org/0000-0003-0747-3890
#Douglas Perry, https://orcid.org/0000-0001-5294-0131

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 with 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 via thrips among artificial habitat patches more readily than fungi. In the field, incidence and abundance of culturable bacteria and fungi were positively correlated, but bacteria were much more widespread. These patterns suggest shared dispersal routes or habitat requirements among culturable bacteria and fungi, but differences in dispersal or colonization frequency by thrips, common flower visitors. The finding that culturable bacteria are more common among nectar sampled here, in part due to superior thrips-mediated dispersal, may have relevance for microbial life history, community assembly of microbes, and plant–pollinator interactions.

Keywords: nectar yeast; plant–microbe; microbial ecology; dispersal; plant–pollinator interactions

Received: 30 June 2021; **Accepted:** 15 November 2021

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 ability, here, defined as realized dispersal, the product of emigration, transport and establishment in a new habitat, is well documented for macroorganisms. Increasing evidence suggests that microbial taxa, including fungi and bacteria, can also be dispersal limited (Lindow and Andersen 1996; Peay *et al*. 2012; Brown and Jumpponen 2014; Albright and Martiny 2018; Svoboda *et al*. 2018). Despite the fact that bacteria and fungi often co-occur within communities, few studies have directly examined their relative dispersal abilities (but see Barberán *et al*. 2015), limiting 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, Hall and Metcalf 2017). Characterizing dispersal differences of microbial taxa under realistic conditions is important to understanding how dispersal may shape microbial community assembly (Schmidt *et al*. 2014).

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, Pozo and Medrano 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, Gauthier and Fukami 2013; Lenaerts *et al*. 2017; Sobhy *et al*. 2018). Bacteria and fungi both frequently 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, Peay and Fukami 2012; Pozo, Lachance and Herrera 2012; Álvarez-Pérez and Herrera 2013) and exhibit traits that may enhance fitness in nectar environments (Álvarez-Pérez, Herrera and de Vega 2012; Pozo, Lachance and Herrera 2012; Dhami, Hartwig and Fukami 2016).

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 2004; 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, Garcia and Perez 2008). Second, in a previous study, taxa detected using culture-based method for bacteria and fungi in nectar largely overlap with those detected using shotgun sequencing (Morris *et al*. 2020). Third, both bacteria and fungi (consisting mainly of yeasts) in nectar are thought to depend on phoresy via flower visitors (Brysch-Herzberg 2004; 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 (Vannette and Fukami 2014; 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 (Álvarez-Pérez and Herrera 2013), so the relative influence of dispersal and species interactions (including competition) on nectar microbe metacommunities is 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.

Here, we test the hypothesis that nectar-inhabiting bacteria and fungi differ in dispersal and occupancy among individual flowers in two coflowering communities. We assess realized dispersal—which integrates the components of emigration (leaving a habitat), survival during transport and establishment in a new habitat (flower)—that ultimately determines community membership (Baguette, Benton and Bullock 2012). First, we examined microbial dispersal ability empirically using thrips, which are common visitors to flowers (Bryan and Smith 1956). Second, we assessed occupancy (incidence) and abundance of culturable bacteria and fungi in individual nectar samples of >1800 flowers, spanning 43 species of plants at two sites to examine the following: (i) do bacteria or fungi differ in dispersal ability by a common flower-visiting insect; (ii) are patterns of bacterial and fungal incidence or abundance in nectar similar across a plant community; (iii) does microbial presence or abundance differ among plant species or with floral accessibility (flower morphology); and (iv) does season, year or site predict patterns of bacterial or fungal incidence and abundance?

MATERIALS AND METHODS

Insect-based microbial dispersal assay

To empirically test whether nectar microbes differ in their dispersal ability, we developed an assay of microbial dispersal resulting from the feeding activity of western flower thrips (*Frankliniella occidentalis*). Thrips are common and abundant flower visitors in all major California plant communities and are associated with at least 139 naturally occurring plant species, 45 plant families and 23 plant orders (Bryan and Smith 1956). Regionally, in the San Francisco Bay Area (within 120 km of study sites), thrips reach densities of up to 400 thrips per 100 flowers, in natural plant communities in the spring (Bryan and Smith 1956). Although morphologically and behaviorally distinct from more commonly studied vectors of microbes to flowers like bees, birds or even beetles, thrips attain high densities in flowers, readily move among flowers and are known vectors of bacteria and viruses, making them likely important vectors of microbes in flowers. Thrips were sourced from a colony held by Diane Ullman (see Fig. S1 and Methods S1, Supporting Information, for assay method details). Before each assay, thrips were sterilized by feeding them a diet containing rifampicin (0.2 mg/mL) and cycloheximide (0.4 mg/mL) for 48 h, followed by vortexing in ethanol (30%) and bleach (5%), followed by a rinsing in sterile water three times (see Fig. S1, Supporting Information). The assay consisted of five sterilized adult female thrips freely

foraging within a 96-well plate in which one-third of wells contained a microbial suspension (200 μL, 10 000 cells/μL) in sweetened tryptic soy broth (TSB; adding 15% sucrose and 15% fructose) and the remaining two-thirds of wells were filled with sterile sweetened TSB (200 μL). Microbial isolates from field sampling in this study (see later and Table S1, Supporting Information) and other nectar-associated microbe collections were used, with separate assays for each strain (six bacteria, five fungi). After thrips foraged for 24 h at 30°C under 16L:8D light, thrips were killed by adding a total of 320 μL of ethyl acetate in 10 μL drops to the space between individual wells on the plate. The media was then incubated for 5 additional days. Control replicates with thrips and no added microbes were included in all trials to ensure adequate sterilization and trial consistency. Following incubation, we assessed microbial occupancy of initially sterile wells by calculating the deviation of optical density (OD_{600nm}) from a blank control plate (no thrips, no microbes) with an occupancy threshold equal to the mean OD of control wells + six standard deviations.

Coflowering community sampling for bacteria and fungi

To assess patterns of microbial incidence and abundance, we sampled standing crop floral nectar during 2016 and 2017 at two sites in northern California: Stebbins Cold Canyon Reserve (38.49806°, –122.09931°, 140 m; hereafter Stebbins) in Winters, CA, and at flowering plots maintained at the Laidlaw Honey Bee Facility (38.53653°, –121.78864°, 19 m; hereafter Bee Biology) in Davis, CA, which hosts an active apiary. The sites are ~30 km apart, and differ in pollinator species composition and anthropogenic influence, but share a subset of plant species. Both sites receive frequent visitation by thrips, honey bees, bumble bees, solitary bees, beetles, ants, butterflies and skippers among other taxa, but we did not systematically quantify visitation rate or composition among plant species.

Between late February and early July, plant communities were sampled every 2–4 weeks. We collected ~10 flowers from each abundant plant species. In some cases, fewer than 10 flowers were collected when a plant's floral abundance was low to protect plant populations. When possible, flowers were sampled from multiple individual plants and subpopulations or plots (Table S2, Supporting Information). Flowers were sampled in late morning, 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 nectar extraction and plating, no more than 5 h later. In the lab, flowers were destructively sampled. Nectar was collected using 10 μL microcapillary tubes, and its volume was quantified. Nectar was diluted in 30 μL of sterile water (D0) so dilutions could be used for other downstream chemical analyses, then diluted 10-fold and 100-fold (D1 and D2, respectively) in sterile phosphate-buffered saline (Dulbecco's; pH 7.1–7.5). 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, BD, Franklin Lakes, NJ, USA) containing the antibacterial chloramphenicol and Reasoner's agar containing the antifungal cycloheximide (R2A Oxoid formula with 16% sucrose, Oxoid, Nepean Ontario, Canada), respectively. All samples were plated on 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 this manuscript although some colonies on each media type may be comprised of microbes resistant to the antimicrobial compounds used here (e.g. bacteria resistant to chloramphenicol; Dhami *et al.* 2018). Based on dilution curves, we were able to detect colony-forming units (CFUs) on plates at ~10^1 live cells in the original nectar sample for YMA and 10^2 cells for R2A (Methods S2 and Fig. S2, Supporting Information).

Negative controls were plated for each sampling bout to detect potential contamination and samples were discarded if contamination was detected (detected for one collection date; these samples were removed from the analysis). Agar plates were incubated at 28°C and CFUs were counted after 48–72 h. 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, 1825 nectar samples were collected and plated on two media types. In 2016, colonies were picked haphazardly from plates collected over the entire season including all sites and plant species and frozen in glycerol.

Nectar accessibility: flower corolla length

Microbial dispersal to nectar is likely affected by the location and accessibility of floral nectar within the flower. Specifically, nectar that is not enclosed by flower tissue may be more easily accessed via wind dispersal and may also be accessible to a greater diversity of insect visitors. On the other hand, nectar protected by flower tissues comprising the perianth (non-reproductive structures of the flower) may be less accessible via wind dispersal and contacted only when flowers are visited by long-tongued birds or insects (Kingston and McQuillan 2000; Lara and Ornelas 2001). We examined if bacterial or fungal incidence or abundance depended on nectar location within the flower (flower corolla length). Nectar within flowers of each species was classified as 'exposed' (no corolla/exposed nectary), or contained within 'short' (1–5 mm), 'mid' (5–15 mm) or 'long' (15+ mm) perianth, based on the corolla tube length (Table S2, Supporting Information).

Microbial identification: culture-based and culture-independent analyses

A subset of microbial strains from glycerol stocks were plated on initial isolation media and identified using matrix-assisted laser desorption/ionization and time of flight mass spectrometry (MALDI-TOF). Briefly, colony material was plated, subcultured, colonies chosen were spotted on a steel plate, extracted with 1 μL of 70% formic acid and overlaid with α-Cyano-4-hydroxycinnamic acid (HCCA) matrix. Spectra were obtained on a Bruker ultrafleXtreme MALDI-TOF and compared with Bruker Bacteria and Eukaryote libraries and a custom in-house database curated from previously identified microbial isolates from nectar (Methods S3, Supporting Information). In addition, we used a culture-independent sequencing approach to characterize diversity in full-length regions of the rRNA genes, including16S for bacteria and internalt transcribed spacer (ITS) sequences from 24 nectar samples, each pooled from an average of 60 flowers in each sample, range of 13–116 flowers per sample, each contributing 2 μL of D0. Pooled samples represented multiple sampling dates at each site from 2016. Pooled nectar samples were extracted using the NucleoSpin Microbial DNA kit (Macherey-Nagel, Bethlehem, PA) and submitted for full-length PacBio16S and ITS sequencing performed at Dalhousie IMR. Recovered sequences were processed using dada2 pipeline

for full-length rRNA genes (Callahan *et al.* 2016, 2019) for recovery of actual sequence variants (ASVs). Briefly, primer sequences were removed, and resulting sequences were quality filtered, error corrected and denoised followed by taxonomy assignment using SILVA v. 138 training set (Quast *et al.* 2012) for bacteria and UNITE 4/2/2020 release for fungi (Kõljalg *et al.* 2005) followed by chimera detection and removal (see Dryad https://doi.org/10.25338/B8403V for bioinformatics scripts). ASVs annotated as chloroplast or plant, and those that were also obtained from extraction blanks were removed. Following removal of chloroplast DNA, insufficient bacterial reads were recovered for analysis but we present a summary of ITS data below, and both are available online.

Statistical analysis

To examine if microbes differed in dispersal ability in our experimental setup, the proportion of wells colonized by a microbe within a plate (calculated using OD as described above; plates as replicates) was analyzed using a binomial GLMM (package lme4) to compare bacterial and fungal dispersal plates with strain as a random effect. A *t*-test was also used to compare bacteria and fungi by the mean proportion of wells colonized by each strain across eight replicate plates.

To examine the relationship between bacterial and fungal incidence and abundance in individual nectar samples, we used Pearson correlations. In addition, we report directly calculated conditional probabilities and a χ^2 test to test for nonindependence of bacterial and fungal incidence amongst flowers.

To compare whether plant species differed in bacterial or fungal presence or abundance, we used logistic regression and linear regression with plant species as a predictor. To compare if nectar accessibility quantified using corolla length category was associated with bacterial or fungal presence or abundance, we used logistic regression and linear regression with nectary location (exposed, short corolla, mid corolla or long corolla) as a predictor.

To determine whether bacteria and fungi differ in incidence in floral nectar with Julian date, between sites or between years, we used a logistic regression implemented using 'glm', using bacteria or fungal presence as a response and site, year and plant species, and their two-way interactions, as predictors. We compared among models containing all interactions, models with nonsignificant interactions removed and models with no interactions using Akaike information criterion (AIC), and from the best-fit model, significance of each term was assessed using likelihood ratio tests. To determine whether bacteria or fungi differ in abundance in floral nectar across years or sites, or with Julian date, we used a linear regression implemented using 'lm', with $\log_{10}(\text{CFUs} + 1)$ as a response and site, Julian date and year as predictors. Model selection was performed on bacteria and fungi separately using AIC as described above, and significance was assessed using likelihood ratio tests.

We assessed the frequency with which microbial genera were detected using MALDI-TOF among flower morphologies, or across the season (early, mid or late; see the Supporting Information for details) using a χ^2 test for contingency tables. Microbial composition from PacBio sequencing is presented to compare taxonomic composition with MALDI-TOF data but due to limited numbers of samples successfully sequenced, no formal analysis was performed. All analyses were conducted using RStudio and R v. 3.6.3.

RESULTS

Insect-based dispersal assay

Fungi were less able to disperse among wells than bacteria in the thrips dispersal assay (Fig. 1). Using strain-level averages to compare dispersal abilities between kingdoms, bacteria colonized 31% more wells than did fungi over 24 h of thrips activity (*t*-test, strains as replicates, $t = 4.31$, $P = 0.002$). Results from the GLMM confirm this difference between fungal and bacterial average dispersal ability (*z*-value $= -4.66$, $P < 0.001$). Control treatments that included thrips had detectable, but low levels of contamination of thrips-associated microbes (Fig. 1) that were identified by Sanger sequencing in preliminary trials. We did not detect microbial growth in plates without thrips or without microbes added in any trial.

Patterns of bacterial and fungal incidence and co-occurrence in floral nectar

Floral nectar samples from the field contained colonies on R2A media (hereafter 'bacteria') in 49% of samples, whereas colonies on YMA (hereafter, 'fungi') were detected in only 20% of samples (microbe LRT $= 21.07$; $P < 0.001$; Fig. 2). Fungi and bacteria co-occur in flowers nearly 1.5 times as frequently as would be expected by chance given their individual incidence across the landscape (14% of flowers contain both, compared with 9% of flowers' estimated joint probability if independent; $\chi^2 = 94.37$, $P < 0.001$). Along with the difference between bacterial (49%) and fungal (20%) incidence across the landscape, this elevated frequency of co-occurrence results in a discrepancy between the likelihoods that fungi or bacteria find themselves co-occupying a flower (conditional probabilities of co-occupancy for each microbe). Fungi (mostly yeasts) are most often found in flowers alongside bacteria (P[bacteria present | fungi present] = 69%). On the other hand, bacteria only occasionally co-occupy a flower with fungi (P[fungi present | bacteria present] = 29%).

Moreover, fungi and bacteria demonstrate a positive correlation in CFU abundance across flowers (Pearson correlation, $r = 0.27$, $P < 0.001$), which persists even when investigating only flowers that contain both bacteria and fungi (Pearson correlation, $r = 0.24$, $P < 0.001$). Within plant species, fungal and bacterial incidence and abundance are most often positively correlated, but only significantly in four species (Fig. S3, Supporting Information).

Nectar location associated with variation in microbial incidence and abundance

Plant species differed in microbial incidence (bacteria LRT = 306, $P < 0.001$; fungi LRT = 271.09, $P < 0.001$) and abundance (bacterial CFUs $F_{41,1816} = 9.3$, $P < 0.001$; fungal CFUs $F_{41,1816} = 7.3$, $P < 0.001$) in floral nectar (Fig. 3) ranging from an average of 20% to 100% of sampled flowers containing bacteria. Nectar location within a flower was associated with some of this variation (Fig. 4). Both bacteria and fungi were more frequently detected in nectar of plant species with an exposed nectary than those with short, mid or long corollas (Fig. 4; bacteria LRT = 8.1, $P = 0.04$; fungi LRT = 52.9, $P < 0.001$). Microbial abundance also varied with flower traits, but bacteria and fungi differed in their responses. Bacteria were more abundant in flowers with exposed nectar ($F_{3,1813} = 4.65$, $P = 0.003$), whereas fungal CFUs were most abundant when nectar is contained within a long corolla (Fig. 4; $F_{3,1810} = 29.1$; $P < 0.001$) compared with other flower morphologies.

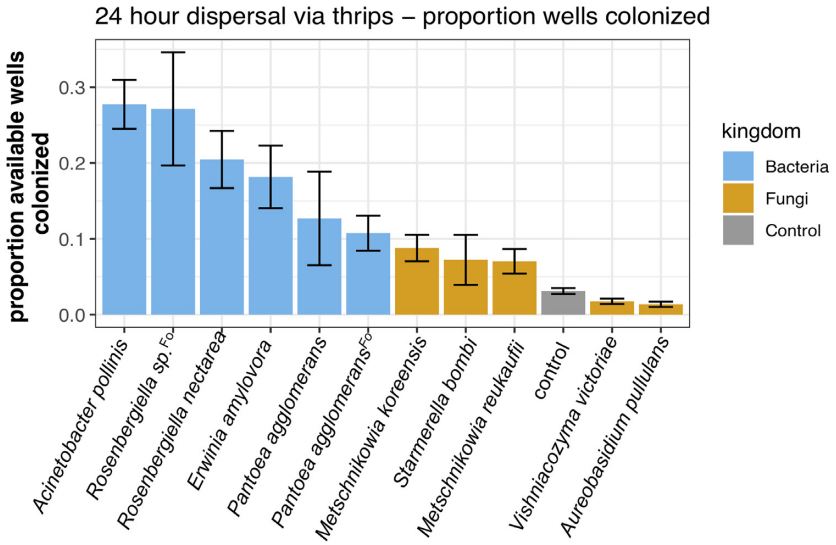

Figure 1. Western flower thrips (*F. occidentalis*) disperse bacteria between artificial nectar habitats more readily than fungi. Each bar shows the relative success of a different isolate of a bacterium or fungus in colonizing wells on a 96-well plate through the activity of sterilized thrips (N = 8–12 plates per isolate). Microbes with superscript 'Fo' were isolated from *F. 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 SE) for the proportion of available wells colonized after 24 h of thrips activity and a subsequent 6 days of culturing in an incubator.

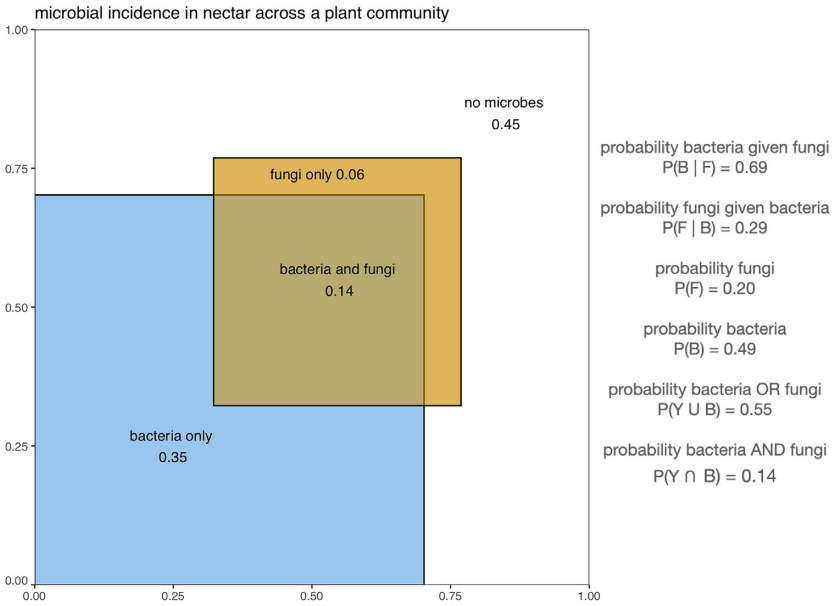

Figure 2. Bacterial and fungal incidence in nectar in the two flowering plant communities sampled. The figure shows the relative incidence of bacteria and fungi in individual nectar according to shaded areas and their proportions of flower occupancy. Bacteria occur in more flowers than fungi across the 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.

Sources of variation in bacterial and fungal incidence and abundance

Microbial incidence differed between geographic sites and years, and changed through the season (Julian date) (Table S3 and Fig. S4, Supporting Information). Bacteria and fungi differed in which site each was more detectable and abundant, with bacteria more frequently detected and abundant at the Bee Biology site (Table S3, Supporting Information) and fungi more frequently detected and abundant at the natural reserve site (Table S3, Supporting Information). The probability of detection of both bacteria and fungi changed across the season—increasing with Julian date in 2017—and across sites relatively similarly (Fig. S4, Supporting Information), whereas patterns of fungal and bacterial abundance tended to differ in response to seasons and years.

Microbial identification and genus-specific patterns

Bacteria from the genus *Acinetobacter* were the most common bacterial taxa detected based on MALDI identification (Table S4 and Fig. S5, Supporting Information). Yeasts from the genera

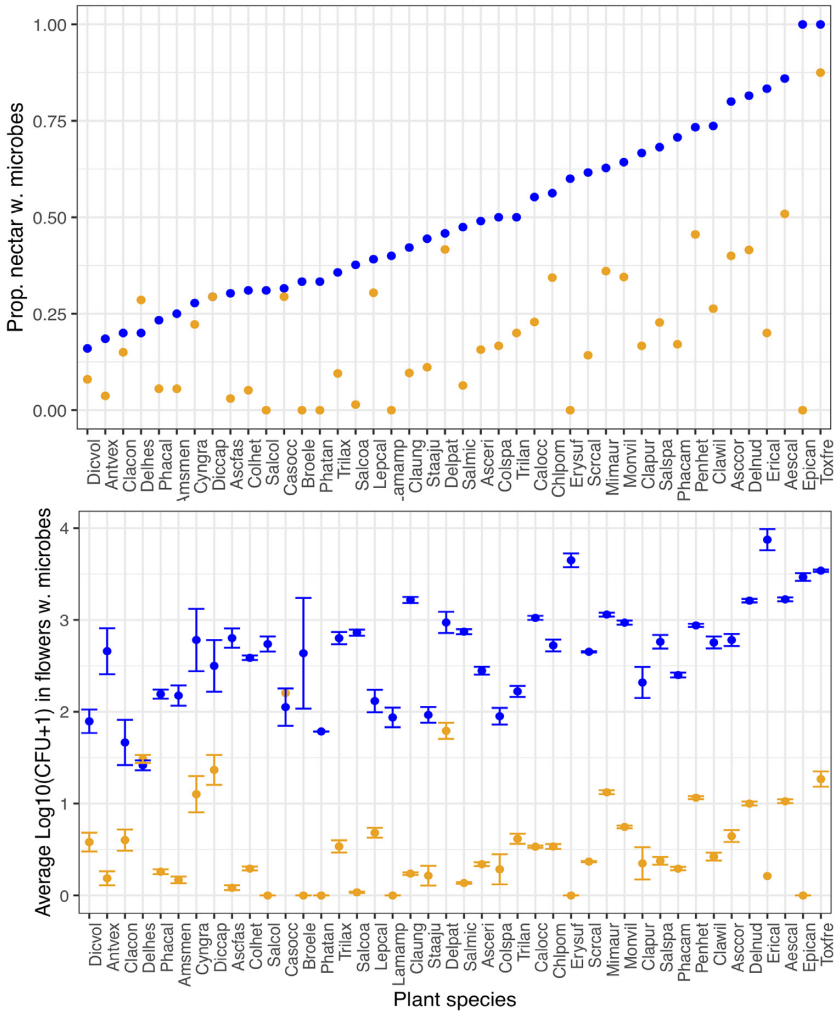

Figure 3. Plant species vary in (top) the proportion of total nectar samples containing colonies or (bottom) the average calculated abundance of CFUs in microbial-colonized nectar on R2A media with antifungal compound (bacteria, blue) or colonies on YMA media with antibacterial (fungi, orange), ordered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

Metschnikowia and *Candida* were the most commonly identified fungi using MALDI and sequencing (Table S4 and Fig. S5, Supporting Information). Filamentous fungi are not able to be identified using MALDI, but *Penicillium*, *Cladosporium* and *Epicoccum* 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 (Table S4, Supporting Information), 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 (Table S5, Supporting Information).

DISCUSSION

In our survey, culturable 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. Although inferring dispersal limitation from incidence data could be biased if habitats (here, nectar from different flowers) vary in suitability for establishment, our results indicate that all plant species can support at least some live bacteria (Fig. 3). We note that incidence estimated using culture-based techniques may be a conservative estimate of incidence and abundance (Methods S2 and Fig. S2, Supporting Information). We emphasize that our conclusions are limited to cultivable taxa, which in some studies align well with culture-independent sequencing results, but we could not assess the extent of bias introduced by culture methods in the current study.

Nectar-inhabiting bacteria and fungi are both likely to rely on and exhibit adaptations for frequent dispersal (Madden *et al.* 2018; Vannette 2020) because individual flowers typically persist for a short time (hours to days) (Primack 1985). Bacteria from the dominant genera found in nectar (e.g. *Acinetobacter* and *Rosenbergiella*) form sticky biofilms (Irie and Parsek 2008; Jung and Park 2015; Álvarez-Pérez, Lievens and Fukami 2019),

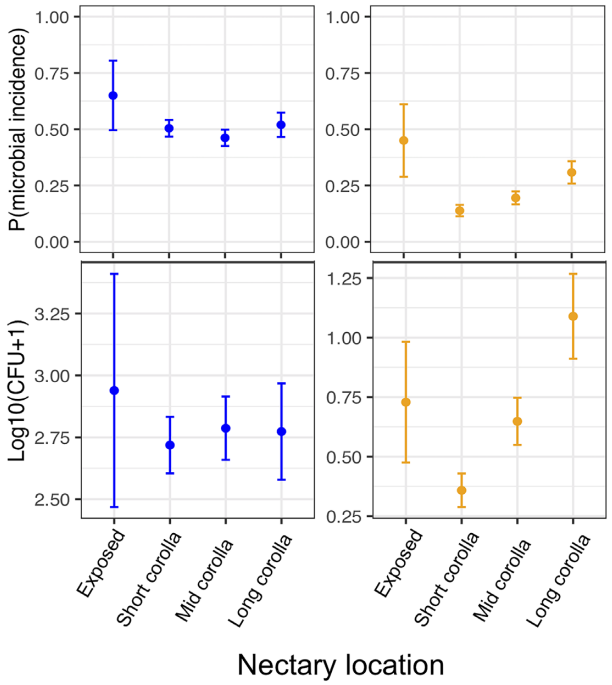

Figure 4. Microbial incidence and abundance in nectar vary by location of the nectar (corolla length). Average microbial abundance in flowers was calculated using CFUs counted on R2A (bacteria, blue) and YMA (fungi, orange) media and adjusted based on nectar volume. Microbial CFU abundance was calculated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

which could enhance adhesion to insect vectors. Yeasts common to nectar exhibit cell morphology that may improve adhesion to insect or bird mouthparts (e.g. 'airplane' cells in the yeast *Metschnikowia gruessii*; Brysch-Herzberg 2004) and produce stress-tolerant spores that enhance desiccation resistance (Francisco *et al.* 2019) and survival during transport between flowers. The emission of microbial volatile compounds (Vannette, Gauthier and Fukami 2013; Rering *et al.* 2018) affects vector attraction to flowers and visit duration (reviewed in Crowley-Gall *et al.* 2021). The greater dispersal ability of bacteria observed here could be due to differential vector attraction (more attractive, less avoided or attracting different organisms); ease of attachment to insect vectors; persistence in association with, survival on or localization within hosts (Perilla-Henao and Casteel 2016); or more efficient colonization success or rapid growth in nectar. Indeed, microbial taxa likely differ in primary vectors. For example, *Metschnikowia* yeasts have been associated with bumble bee vectors in previous work (Herrera, Pozo and Medrano 2013; Jacquemyn *et al.* 2021). In contrast, thrips, long ignored as potential microbial vectors among flowers, are also competent vectors of nectar microbes, particularly bacteria. Given their frequent high abundance in flowers, thrips' role in shaping nectar microbe communities may be important but currently overlooked. Dispersal via wind, water or even plant vascular tissue may be possible for some microbial taxa, but yeasts are rare in airborne samples (Last and Price 1969) and both fungi and bacteria may require specific adaptations for airborne dispersal. It is likely that variation in floral visitor composition, abundance and microbial communities vectored can explain variation in nectar microbial composition within individual plants and communities (Brysch-Herzberg 2004; de Vega *et al.* 2021; Zemenick, Vannette and Rosenheim 2021).

Not only dispersal, but also variation in flower characteristics that affect microbial growth could affect the patterns observed in our field study (Figs 3 and 4). In some plant species, floral nectar contains antimicrobial compounds (González-Teuber and Heil 2009), including antifungal and antibacterial enzymes (Thornburg *et al.* 2003; Carter and Thornburg 2004; Roy *et al.* 2017; Schmitt *et al.* 2018), some of which could differentially reduce bacterial or fungal growth. Other floral traits, including corolla length, nectar production rate, UV exposure (Plowright 1987) and flower longevity, could differentially affect yeast and bacterial establishment, growth and interactions between them.

Distinct patterns of bacterial and fungal presence and abundance in floral nectar documented here have a few specific implications for the ecology and evolution of the nectar microbiome. For more prevalent nectar bacteria, interkingdom competition may be less important in the evolution of bacterial traits and life history than for fungi, which are frequently exposed to competition with bacteria (Fig. 2), but experimental work is lacking. In contrast, experimental and genomic evidence suggests that *Metschnikowia reukaufii* benefits from rapid nutrient assimilation and population growth (Pozo, Lachance and Herrera 2012; Dhami, Hartwig and Fukami 2016), suggesting that maximizing propagule abundance is an important strategy for nectar microbial populations (Hibbing *et al.* 2010). Our data do not reveal negative correlations between fungi and bacterial abundance in nectar, either among or within species (Fig. S3, Supporting Information), suggesting that interference competition may not be strong in our focal populations. Instead, positive correlations between fungal and bacterial abundance at the flower level could indicate shared dispersal vectors, similar habitat requirements for culturable nectar microbes or the possibility of facilitation that could be promoted by bacterial enhancement of nutrients in nectar from pollen or other plant sources (Christensen, Munkres and Vannette 2021).

Here, we show experimentally that thrips more effectively vector bacteria compared with yeasts and the survey demonstrates that fungi are less frequently detected in flowers. Our results suggest that bacteria and fungi differ in dispersal probability via a common insect vector, although we caution that this difference likely depends on the particular flower visitor species examined. Previous work has shown that dispersal can be important in structuring bacterial community assembly (Albright and Martiny 2018), and generates the possibility of historically contingent competitive outcomes in microbial communities (Fukami 2015; Svoboda *et al.* 2018). 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 interactions in flowers.

ACKNOWLEDGMENTS

We are grateful to the University of California Natural Reserve System, the Laidlaw Honey Bee Research Facility, Jeffrey Clarey and Neal Williams for site access, Diane Ullman for the thrips colony, Amin Montazer for help with lab work, Will Jewell and Barbara Byrnes for training and technical support with MALDI-TOF and the Vannette lab group for providing feedback on initial drafts of this manuscript.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

FUNDING

RLV was supported by UC Davis, the Hellman Fund, the United States Department of Agriculture (USDA) Hatch funds multistate (NE1501) and the National Science Foundation (DEB #1846266). MSM was supported by the USDA—National Institute of Food and Agriculture grant no. 2020-67034-31942.

Conflict of interest. None declared.

DATA AND CODE ACCESSIBILITY STATEMENT

The data underlying this article are available in NCBI Short Read Archive at https://www.ncbi.nlm.nih.gov/sra/PRJNA727353, and all data and code are available through Dryad (https://doi.org/10.25338/B8403V).

AUTHOR CONTRIBUTIONS

RLV conceived of the field study, performed fieldwork and wrote the manuscript. MSM and RLV conceived of the thrips dispersal assay and performed statistical analyses. GWH performed field and microbial lab work. MSM and DP performed the thrips dispersal assay. IM analyzed and processed samples for MALDI-TOF identifications. TGM performed dilution plating assays. All authors contributed to revisions and gave final approval for publication.

REFERENCES

Albright MBN, Martiny JBH. Dispersal alters bacterial diversity and composition in a natural community. *ISME J* 2018;**12**:296–9.

Alexander HM, Antonovics J. Disease spread and population dynamics of anther-smut infection of *Silene alba* caused by the fungus *Ustilago violacea*. *J Ecol* 1988;**76**:91–104.

Álvarez-Pérez S, Herrera CM. Composition, richness and nonrandom assembly of culturable bacterial–microfungal communities in floral nectar of Mediterranean plants. *FEMS Microbiol Ecol* 2013;**83**:685–99.

Álvarez-Pérez S, Herrera CM, de Vega C. Zooming-in on floral nectar: a first exploration of nectar-associated bacteria in wild plant communities. *FEMS Microbiol Ecol* 2012;**80**:591–602.

Álvarez-Pérez S, Lievens B, Fukami T. Yeast–bacterium interactions: the next frontier in nectar research. *Trends Plant Sci* 2019;**24**:393–401.

Baguette M, Benton TG, Bullock JM. *Dispersal Ecology and Evolution*. Oxford: Oxford University Press, 2012.

Barberán A, Ladau J, Leff JW *et al*. Continental-scale distributions of dust-associated bacteria and fungi. *Proc Natl Acad Sci USA* 2015;**112**:5756–61.

Belisle M, Peay KG, Fukami T. Flowers as islands: spatial distribution of nectar-inhabiting microfungi among plants of *Mimulus aurantiacus*, a hummingbird-pollinated shrub. *Microb Ecol* 2012;**63**:711–8.

Brown SP, Jumpponen A. Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. *Mol Ecol* 2014;**23**:481–97.

Bryan D, Smith R. The *Frankliniella occidentalis* (Pergande) complex in California (Thysanoptera: Thripidae). *Univ Calif Publ Entomol* 1956;**10**:359–410.

Brysch-Herzberg M. Ecology of yeasts in plant–bumblebee mutualism in Central Europe. *FEMS Microbiol Ecol* 2004;**50**:87–100.

Callahan BJ, McMurdie PJ, Rosen MJ *et al*. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581.

Callahan BJ, Wong J, Heiner C *et al*. High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. *Nucleic Acids Res* 2019;**47**:e103.

Carter C, Thornburg RW. Is the nectar redox cycle a floral defense against microbial attack? *Trends Plant Sci* 2004;**9**:320–4.

Chappell CR, Fukami T. Nectar yeasts: a natural microcosm for ecology. *Yeast* 2018;**35**:417–23.

Christensen SM, Munkres I, Vannette RL. Nectar bacteria stimulate pollen germination and bursting to enhance microbial fitness. *Curr Biol* 2021;**31**:4373–80.e6.

Crowley-Gall A, Rering CR, Rudolph AB *et al*. Volatile microbial semiochemicals and insect perception at flowers. *Curr Opin Insect Sci* 2021;**44**:23–34.

de Vega C, Álvarez-Pérez S, Albaladejo RG *et al*. The role of plant–pollinator interactions in structuring nectar microbial communities. *J Ecol* 2021;**109**:3379–95.

Dhami MK, Hartwig T, Fukami T. Genetic basis of priority effects: insights from nectar yeast. *Proc Biol Sci* 2016;**283**:20161455.

Dhami MK, Hartwig T, Letten AD *et al*. Genomic diversity of a nectar yeast clusters into metabolically, but not geographically, distinct lineages. *Mol Ecol* 2018;**27**:2067–76.

Francisco CS, Ma X, Zwyssig MM *et al*. Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. *Sci Rep* 2019;**9**:9642.

Fridman S, Izhaki I, Gerchman Y *et al*. Bacterial communities in floral nectar. *Environ Microbiol Rep* 2012;**4**:97–104.

Fukami T. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annu Rev Ecol Evol Syst* 2015;**46**:1–23.

González-Teuber M, Heil M. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal Behav* 2009;**4**:809–13.

Herrera CM, Canto A, Pozo MI *et al*. Inhospitable sweetness: nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. *Proc Biol Sci* 2010;**277**:747–54.

Herrera CM, de Vega C, Canto A *et al*. Yeasts in floral nectar: a quantitative survey. *Ann Bot (Lond)* 2009;**103**:1415–23.

Herrera CM, Garcia IM, Perez R. Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology* 2008;**89**:2369–76.

Herrera CM, Pozo MI, Medrano M. Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. *Ecology* 2013;**94**:273–9.

Hibbing ME, Fuqua C, Parsek MR *et al*. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 2010;**8**:15–25.

Irie Y, Parsek MR. Quorum sensing and microbial biofilms. In: Romeo T (ed). *Bacterial Biofilms*. Berlin, Heidelberg: Springer, 2008, 67–84.

Jacquemyn H, Pozo MI, Álvarez-Pérez S *et al*. Yeast–nectar interactions: metacommunities and effects on pollinators. *Curr Opin Insect Sci* 2021;**44**:35–40.

Jung J, Park W. *Acinetobacter* species as model microorganisms in environmental microbiology: current state and perspectives. *Appl Microbiol Biotechnol* 2015;**99**:2533–48.

Kingston AB, McQuillan PB. Are pollination syndromes useful predictors of floral visitors in Tasmania? *Austral Ecol* 2000;**25**:600–9.

Kõljalg U, Larsson KH, Abarenkov K *et al*. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 2005;**166**:1063–8.

Koskella B, Hall LJ, Metcalf CJE. The microbiome beyond the horizon of ecological and evolutionary theory. *Nat Ecol Evol* 2017;**1**:1606–15.

Lachance MA, Starmer WT, Rosa CA *et al*. Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Res* 2001;**1**:1–8.

Lara C, Ornelas J. Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. *Oecologia* 2001;**128**:263–73.

Last FT, Price D. Yeast associations with living plants and their environs. In: Rose AH, Harrison JS (eds). *The Yeasts*. New York: Academic Press, 1969, 183–218.

Leibold MA, Holyoak M, Mouquet N *et al*. The metacommunity concept: a framework for multi-scale community ecology. *Ecol Lett* 2004;**7**:601–13.

Lenaerts M, Goelen T, Paulussen C *et al*. Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. *Funct Ecol* 2017;**31**:2061–9

Lindow SE, Andersen GL. Influence of immigration on epiphytic bacterial populations on navel orange leaves. *Appl Environ Microbiol* 1996;**62**:2978–87.

Madden AA, Epps MJ, Fukami T *et al*. The ecology of insect–yeast relationships and its relevance to human industry. *Proc Biol Sci* 2018;**285**:20172733.

Mittelbach M, Yurkov AM, Stoll R *et al*. Inoculation order of nectar-borne yeasts opens a door for transient species and changes nectar rewarded to pollinators. *Fungal Ecol* 2016;**22**:90–7

Morris M, Frixione N, Burkert A *et al*. Microbial abundance, composition, and function in nectar are shaped by flower visitor identity. *FEMS Microbiol Ecol* 2020;**96**:3.

Mouquet N, Loreau M. Community patterns in source-sink metacommunities. *Am Nat* 2003;**162**:544–57.

Ngugi HK, Scherm H. Biology of flower-infecting fungi. *Annu Rev Phytopathol* 2006;**44**:261–82.

Peay KG, Schubert MG, Nguyen NH *et al*. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Mol Ecol* 2012;**21**:4122–36.

Perilla-Henao LM, Casteel CL. Vector-borne bacterial plant pathogens: interactions with hemipteran insects and plants. *Front Plant Sci* 2016;**7**:1163.

Plowright R. Corolla depth and nectar concentration: an experimental study. *Can J Bot* 1987;**65**:1011–3.

Pozo MI, Lachance M-A, Herrera CM. Nectar yeasts of two southern Spanish plants: the roles of immigration and physiological traits in community assembly. *FEMS Microbiol Ecol* 2012;**80**:281–93.

Primack RB. Longevity of individual flowers. *Annu Rev Ecol Syst* 1985;**16**:15–37.

Quast C, Pruesse E, Yilmaz P *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012;**41**:D590–6.

Rering CC, Beck JJ, Hall GW *et al*. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytol* 2018;**220**:750–9

Roy R, Schmitt AJ, Thomas JB *et al*. Nectar biology: from molecules to ecosystems. *Plant Sci* 2017;**262**:148–64.

Schaeffer R, Irwin RE. Yeasts in nectar enhance male fitness in a montane perennial herb. *Ecology* 2014;**95**:1792–8.

Schmitt AJ, Sathoff AE, Holl C *et al*. The major nectar protein of *Brassica rapa* is a non-specific lipid transfer protein, BrLTP2.1, with strong antifungal activity. *J Exp Bot* 2018;**69**:5587–97.

Schmidt SK, Nemergut DR, Darcy JL *et al*. Do bacterial and fungal communities assemble differently during primary succession? *Mol Ecol* 2014;**23**:254–8.

Sobhy IS, Baets D, Goelen T *et al*. Sweet scents: nectar specialist yeasts enhance nectar attraction of a generalist aphid parasitoid without affecting survival. *Front Plant Sci* 2018;**9**:1009.

Svoboda P, Lindström ES, Osman OA *et al*. Dispersal timing determines the importance of priority effects in bacterial communities. *ISME J* 2018;**12**:644–6.

Thornburg RW, Carter C, Powell A *et al*. A major function of the tobacco floral nectary is defense against microbial attack. *Plant Syst Evol* 2003;**238**:211–8.

Toju H, Vannette RL, Gauthier MPL *et al*. Priority effects can persist across floral generations in nectar microbial metacommunities. *Oikos* 2018;**127**:345–52.

Tsuji K, Fukami T. Community-wide consequences of sexual dimorphism: evidence from nectar microbes in dioecious plants. *Ecology* 2018;**99**:2476–84.

Tucker CM, Fukami T. Environmental variability counteracts priority effects to facilitate species coexistence: evidence from nectar microbes. *Proc Biol Sci* 2014;**281**:20132637.

Vannette RL. The floral microbiome: plant, pollinator, and microbial perspectives. *Annu Rev Ecol Evol Syst* 2020;**51**:363–86.

Vannette RL, Fukami T. Historical contingency in species interactions: towards niche-based predictions. *Ecol Lett* 2014;**17**:115–24.

Vannette RL, Fukami T. Dispersal enhances beta diversity in nectar microbes. *Ecol Lett* 2017;**20**:901–10

Vannette RL, Gauthier M-PL, Fukami T. Nectar bacteria, but not yeast, weaken a plant–pollinator mutualism. *Proc Biol Sci* 2013;**280**:20122601.

von Arx M, Moore A, Davidowitz G *et al*. Diversity and distribution of microbial communities in floral nectar of two night-blooming plants of the Sonoran Desert. *PLoS One* 2019;**14**:e0225309.

Zemenick AT, Vannette RL, Rosenheim JA. Linked networks reveal dual roles of insect dispersal and species sorting for bacterial communities in flowers. *Oikos* 2021;**130**:697–707.