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The Floral Microbiome: Plant, Pollinator, and Microbial Perspectives

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Abstract

Flowers at times host abundant and specialized communities of bacteria and fungi that influence floral phenotypes and interactions with pollinators. Ecological processes drive variation in microbial abundance and composition at multiple scales, including among plant species, among flower tissues, and among flowers on the same plant. Variation in microbial effects on floral phenotype suggests that microbial metabolites could cue the presence or quality of rewards for pollinators, but most plants are unlikely to rely on microbes for pollinator attraction or reproduction. From a microbial perspective, flowers offer opportunities to disperse between flowers, but microbial species differ in requirements for and benefits received from such dispersal. The extent to which floral microbes shape the evolution of floral traits, influence fitness of floral visitors, and respond to anthropogenic change is unclear. A deeper understanding of these phenomena could illuminate the ecological and evolutionary importance of floral microbiomes and their role in the conservation of plant–pollinator interactions.

INTRODUCTION

Pollination is most frequently viewed as a bipartite interaction between plants and pollinators. However, it is now clear that many other organisms influence the ecology and evolution of plant–pollinator interactions (Strauss & Whittall 2006). Flowers frequently contain abundant microbial populations, which can influence pollination in many ways. From what we know of floral microbes, these organisms have their own fascinating ecologies, which reflect their ability to colonize ephemeral flowers, associate with flower visitors including effective pollinators, and metabolize unique nutrient sources in flowers. In this review, I describe current understanding of which, when, and how microbial communities form on and within flowers; how microbes affect floral phenotypes and plant–pollinator interactions; and how microbes disperse out of flowers. I suggest that a defining feature of the floral microbiome is its variability, which generates specific consequences for both flowering plants and flower visitors. I conclude by outlining promising areas for future study of the floral microbiome, with specific reference to pollinator conservation and agricultural sustainability.

HISTORICAL INTEREST IN THE FLORAL MICROBIOME

A microbiome is the community of bacteria, fungi, protozoa, viruses, and phage in a given environment. Hereafter, I use the term microbes to refer to organisms within this community. Within flowers, bacteria and fungi have received the most study and therefore are the main subjects of this review.

Interest in the flower microbiome has recently intensified, but flower microbes have been studied for over a century. One of the best-studied flower-inhabiting microbes is the bacterium *Erwinia amylovora*. Native to North American flowering trees in the family Rosaceae (which includes many commercially important fruits), *E. amylovora* spread rapidly to the related species of apple, pear, and quince soon after their seventeenth-century introduction to the continent (Schroth et al. 1974). The bacterium infected trees through flowers and other open vascular tissues, causing a disease known as fire blight that is characterized by rapid limb browning and tree death. Although rain events and insect visitation to flowers were known to be associated with spread of fire blight among trees, the cause of the disease remained elusive. The 1877 discovery that *E. amylovora* caused fire blight established for the first time that bacteria were able to produce disease in plants (Schroth et al. 1974, Vanneste 2000). Despite identification of its cause, fire blight was not easily controlled. Unscrupulous agents sold remedies for fire blight, leading the Missouri Agricultural Experiment Circular to declare that "driving rusty iron nails intro trees and hanging horse shoes among the branches will not control fire blight" (Talbert 1925, p. 8) Fire blight continued to cause widespread damage in the eastern United States and decimated pear production on the West Coast into the 1900s. Since that time, fire blight has spread worldwide and continues to cost hundreds of millions of US dollars in microbe control and crop losses yearly in apples, pears, and their botanical relatives and to limit the geographical extent of their cultivation (Schroth et al. 1974, Vanneste 2000).

The past few decades have seen exciting progress in characterizing the microbiome of flowers in greater breadth and depth than was previously possible, as is true for microbiomes generally. Multiple disciplines have contributed to the growing interest in floral microbiomes. In the search for novel yeast species, flowers and their insect visitors have been recognized as rich sources of biodiversity (Brysch-Herzberg 2004, Lachance et al. 2001). In community ecology, nectar microcosms have been used to examine drivers of community assembly (Chappell & Fukami 2018, Herrera et al. 2010, Peay et al. 2012). In conservation biology, pollinator declines and increasing recognition of the role of pathogens have led to the identification of flowers as transmission

sites for important pollinator pathogens (Durrer & Schmid-Hempel 1994, Figueroa et al. 2019). In plant pathology, floral surfaces have yielded biocontrol agents for floral pathogens, enhancing sustainable pathogen control in orchards (Lindow & Suslow 2003, Pusey 1999). Together, these disparate studies show that the floral microbiome both is a useful model system for the study of ecology and evolution and could have wide-ranging effects on the ecology and evolution of flowering plants and animals that visit flowers.

WHEN AND HOW DO MICROBES ARRIVE TO FLOWERS?

Newly opened flowers are not sterile. Microbes are nearly always present on at least some flower tissues. But flowers vary, often substantially, in the abundance and composition of microbial communities. Many methods can determine microbial presence and identity, and each yields different types of information subject to different bias (**Table 1**). Nevertheless, surveys of flowers largely converge on the following three generalities about flower microbial communities.

First, bacteria and fungi are present early in a flower's life. Even before anthesis (flower opening), flower buds and nectar in unopened flowers can contain detectable bacterial and fungal species (Shade et al. 2013, von Arx et al. 2019). Petals of newly opened flowers contain detectable, culturable microbial communities (Junker et al. 2011). Flower stigmas and hypanthia (the floral cup containing the nectar) of newly opened apple blossoms also yield culturable bacteria and fungi, although microbial incidence can be low, with 8–35% of samples yielding microbial growth at anthesis (Pusey et al. 2009). Filamentous fungi can be isolated or detected within ovaries in grasses (Hinton & Bacon 1985) and in the pollen of forbs (Hodgson et al. 2014) early in floral development.

Second, microbial incidence and abundance increase over time on individual flowers. The clearest demonstrations come from characterization of microbes inhabiting nectar and the surface of the pistil. In the night-blooming plant species *Datura wrightii* and *Agave palmeri*, bacterial and fungal colony-forming units in nectar were found in low abundance in preanthesis flowers and then increased exponentially over time (von Arx et al. 2019). In the hummingbird-pollinated flower *Mimulus aurantiacus*, yeasts were detected in the nectar of 20% of 1-day-old flowers sampled and between 60 and 80% of older (3- to 6-day-old) flowers (Peay et al. 2012). Similarly, bacterial incidence and abundance increased over time in nectar of the shrub *Epilobium canum* (Morris et al. 2020) and on stigmas and hypanthia of apple blossoms (Pusey et al. 2009). In a whole-flower survey of apple flowers, sequence-read abundance (an imperfect proxy for microbial abundance) increased with flower age, at least early in flower life span (Shade et al. 2013). Not all studies of flower microbiomes examine change in microbial abundance over time, and data are notably missing from most studies of petal and pollen microbiomes. To investigate if this pattern is common across flower tissues or plant species, future studies should take care to use methods that assess microbial abundance (e.g., quantitative polymerase chain reaction, microscopic examination, or other quantitative methods; see **Table 1**).

Third, animal visitation to flowers alters floral microbiomes. Although microbes can be present on flowers that have apparently not been visited, abundant evidence supports a role for animal visitors as major vectors of bacteria, fungi, and other microorganisms to and among flowers. As early as 1884, Boutroux documented the increased presence of fermentative yeasts in flowers that had been visited by bees compared to those not visited (Boutroux 1884). More recent work demonstrates that flowers do not contain ascomycete yeasts when bees and large-bodied pollinators are excluded, but these yeasts are abundant in nectar of pollinator-visited flowers (Belisle et al. 2012; Herrera et al. 2008, 2010). Specific pollinator species can be implicated in dispersal among flowers in some cases (Brysch-Herzberg 2004, Lachance et al. 2001). In

Table 1 Commonly used techniques to characterize the floral microbiome, along with benefits and drawbacks associated with each method

Technique	Cell culture and plating	Metabarcoding (amplicon sequencing)	Visualization	Shotgun metagenomics
Definition	Isolating living microorganisms on media plates in the laboratory	Sequencing short barcode regions to characterize bacterial or fungal communities	Visualization using light microscopy, electron microscopy, or other methods	Untargeted sequencing of DNA within a sample
Benefits	Many floral bacteria and yeasts are culturable on standard lab media (Morris et al. 2020) Allows relative quantification of living cells Allows taxonomic, genomic, and phenotypic description of living organisms Living organisms can be archived and used for experiments	Identifies microbial strains to genus or in some cases species, including those not culturable using standard lab media High throughput, allowing characterization of entire communities in a single sample Standard methods and sequence data formats allow comparison among studies	Allows for quantification of microbial cells and may inform localization within flowers	Can reveal taxonomy and functional potential of bacteria, fungi, and other organisms in the same run Avoids most primer bias Can recover high-quality genomes of abundant organisms that enable taxa-function pairing
Drawbacks	Not all microbes are readily culturable Many media types may be necessary to characterize communities adequately Relatively low throughput; colonies must be individually identified using Sanger sequencing or other methods	Low microbial biomass on individual flowers, so polymerase chain reaction–based approaches can be subject to contamination Does not quantify microbial cells Sequencing yields proportional data No information on microbial function Differences among microbial species in DNA extraction efficiency, primer biases, or sequencing can affect estimates of species composition and relative abundance	Additional staining necessary to reveal if cells are alive or dead Probes or specialized methods are required for microbial identification	More costly than amplicon sequencing, particularly for high coverage Relies on assembly or annotation of short reads For some flower tissues, high abundance of host plant DNA may be problematic

the hummingbird-pollinated species *Mimulus aurantiacus* and *Epilobium canum*, nectar bacteria are detected more frequently and in higher abundance in flowers visited by hummingbirds or large insects (Morris et al. 2020, Vannette & Fukami 2017). Nitidulid beetles vector particular species of large-spored ascomycete yeasts (Lachance et al. 2001), and ants, florivores, and other nonmutualistic floral visitors have been shown to disperse flower-colonizing bacteria and yeasts

(de Vega & Herrera 2013, Samuni-Blank et al. 2014). Small insects may be important but unrecognized vectors of nectar microbes. For example, microbiomes of thrips comprise the bacterial genera *Rosenbergiella* and *Pantoea* (Chanbusarakum & Ullman 2008), which are also isolated from preanthesis flowers (von Arx et al. 2019) and pollen (Manirajan et al. 2016).

Although most evidence that microbial dispersal depends on animal vectors comes from nectar microbes, floral visitors also influence microbial communities on petals, stigmas, pollen, and other parts of the flower. Bumble bees deposit bacteria mainly on the petals and stamens (Russell et al. 2019). Bee feces that contain microbes are frequently detected on flower tissues, including within the corolla and outside the corolla, and on surrounding bracts and nearby leaves. The location of feces varies depending on flower morphology and bee species (Figueroa et al. 2019). Insects have been implicated in the species composition of the pollen microbiome (Manirajan et al. 2016), but further study is necessary to confirm the importance of vectors for pollen microbes.

WHICH MICROBES ARE FOUND ON FLOWERS?

The microbes that are found on flowers can come from a few different sources (**Table 2**). Bacteria and fungi that are commonly found in air, soil, and other habitats can be detected on flower buds and early-stage petals (Morris et al. 2020, Shade et al. 2013). These environmentally common microbes are among the first to be detected once the flower opens but typically do not attain high abundance in flowers (Brysch-Herzberg 2004, Morris et al. 2020).

Microbes from a plant's vegetative tissues can also colonize its flowers (**Table 2**). Plant endophytes (bacteria or fungi that grow asymptomatically within plant tissues), pathogens, and phyllosphere microbes (which colonize plant surfaces) can colonize floral tissues via multiple routes. Fungal hyphae have been visualized growing from vegetative parts to flower tissue in grasses (Hinton & Bacon 1985). Bacteria can also travel within plant vascular tissues, including from flowers to vegetative or reproductive tissues (An et al. 2020, Kim et al. 2019, Vanneste 2000). Specialized xylem-dwelling bacteria can be exuded from water pores (hydathodes) (An et al. 2020), suggesting that exudation into nectar may also be possible depending on nectary type (Roy et al. 2017), but no evidence for this specific phenomenon has been published to date. Plant epiphytes disperse from leaves to flowers, or from another common source to both leaves and flowers, by wind, flowing water, rain droplets, or arthropods that traverse vegetative and floral tissues. Studies that compare epiphytic bacterial communities on leaves to those on petals or whole-flower tissues find similar bacterial species composition (Junker et al. 2011, Massoni et al. 2019, Wei & Ashman 2018), but flower communities are often characterized by lower species richness and evenness than leaves. Evidence from these studies is consistent with movement of microbe populations on leaves to flowers or to both from a shared source pool. Dispersal of microbes from flowers to the leaf phyllosphere is also possible but may be less common due to the shorter life span of flowers. Although leaves and flowers are often similar in microbial species composition, it is unclear if microbial strains differ between habitats. Insights into microbial source pools and microbial adaptation to life on flowers could be gained from individual cell lineage tracking or genomic comparisons between flower and leaf isolates.

Another group of bacteria and fungi is common and can become abundant in flowers but is not typically found on leaves or other plant environments. Here, I refer to these microbes as flower specialists (**Table 2**), although they may be referred to elsewhere as autochthonous microbes (Lachance et al. 2001). Flower specialists are also detected in the crop (honey stomach) of bees and in stored pollen, but the abundance of flower specialists is typically low outside of flowers (Brysch-Herzberg 2004, Pozo et al. 2012). Although the strongest evidence of habitat specialization of floral specialists is their limited occurrence outside of nectar, flower specialist microbes

Table 2 Groups of microbes found in flowers

Groups of microbes found on or in flowers (definition)	Example bacterial genera	Example fungal genera	Characteristics	Effects on floral phenotype	Flower tissues	Microbial abundance on flowers (maximum)	Reliance on vectoring by animals	Reference(s)
Environmental (wind or water dispersed)	*Micrococcus*, TM7	*Aspergillus*, *Penicillium*	Spore forming or environmentally tolerant	Unknown	All flower tissues	Low	Low	Shade et al. 2013
Plant phyllosphere (found on plant surfaces)	*Pseudomonas*, *Sphingomonas*	*Aureobasidium pullulans*, *Cryptococcus* spp., *Rhodotorula mucilaginosa*	Stress tolerant, produce plant hormones, biofilm producing	Metabolize plant compounds, produce volatiles or other metabolic by-products	Petals	High	Low	Farré-Armengol et al. 2016, Junker et al. 2011, Pozo et al. 2012
Plant endophytes (including entomopathogenic microbes and plant pathogens, viruses)	*Erwinia*, *Xanthomonas*, *Streptomyces*	*Alternaria*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Epichloë*, *Microbotryum*, *Monilinia*	Able to colonize plant tissues asymptomatically or symptomatically	Can increase attractiveness; some can produce flower mimics on plants	Petals, pollen, pistils, nectar	Variable: typically low, but can be high	Variable (low–high)	An et al. 2020, Groen et al. 2016, Kim et al. 2019
Flower specialists (flowers are primary habitat)	*Acinetobacter*, *Rosenbergiella*	*Metschnikowia reukaufii*, *Metschnikowia gruessi*	Predominantly isolated from flowers or flower-related habitats; numerically dominant in flowers when present	Abundant growth and metabolism of nectar and floral resources	Nectar, pollen, pistils	Often high when present	High	Alvarez-Perez et al. 2012, Brysch-Herzberg 2004, Herrera et al. 2008, Schaeffer & Irwin 2014, Yang et al. 2019

(*Continued*)

Table 2 (*Continued*)

Groups of microbes found on or in flowers (definition)	Example bacterial genera	Example fungal genera	Characteristics	Effects on floral phenotype	Flower tissues	Microbial abundance on flowers (maximum)	Reliance on vectoring by animals	Reference(s)
Animal-associated microbes (commensal or beneficial)	*Asaia*, *Lactobacillus*	*Kodamaea*, *Metschnikowia*, *Starmerella*, *Wickerhamiella*, (some species)	Found both in flowers and animals that use floral resources (e.g., nectar, pollen); often isolated but not frequently numerically dominant in flowers	Some can grow and metabolize floral resources, others may have short lifespan on flowers	Petals, nectar, pollen	Variable	High	Brysch-Herzberg 2004, Lachance et al. 2001, McFrederick et al. 2012
Animal pathogens (detrimental to flower visitors)	*Paenibacillus*, *Salmonella*, *Serratia*, *Spiroplasma*	*Ascosphaera*, *Aspergillus*	Often deposited on flowers via defecation; often have a short life span on floral tissues	Short-lived, minimal effects on plant phenotype, but pollinators may be able to detect	Petals and sepals, pollen, nectar, stigma	Low	High	Fouks & Lattorff 2011; reviewed by McArt et al. 2014

also exhibit a range of traits hypothesized to be adaptations to floral or nectar environments, including nutrient scavenging and rapid growth (Dhami et al. 2016), ability to survive high and varying osmotic conditions (Herrera et al. 2010), and ability to metabolize flower-specific resources (Lachance et al. 2001, Pozo & Jacquemyn 2019). However, comparative and experimental studies are required to examine which microbial traits are adaptations to living in flowers or particular flower tissues (Pozo et al. 2012).

Animal-associated microbes are frequently found on flowers (**Table 2**). These bacteria, fungi, and viruses, which can also be isolated from animal digestive tracts (Corby-Harris et al. 2014), mouthparts (Belisle et al. 2012, Pozo et al. 2012), or colony or natal environments (Brysch-Herzberg 2004, McFrederick et al. 2012, 2017), may be beneficial, commensal, or pathogenic to pollinators or other floral visitors. Typically, these microbes do not attain high abundance on flowers (Lachance et al. 2001) but can survive at least temporarily on floral surfaces, in some cases long enough to encounter a new animal host. Although experiments demonstrate that pollinators can be infected by pathogens after floral contact, the importance of flowers compared to other locations for transmission of animal-associated microbes to new hosts requires further study.

Microbial life history and niche breadth (**Table 2**) can be difficult to assign for a few reasons. The short rRNA regions frequently used in amplicon sequencing cannot distinguish bacterial or fungal species or strains, so ecologically distinct taxa can appear identical at this barcoding region (Dhami et al. 2018). Moreover, microbial detection in the environment is difficult, so determining where microorganisms are (and are not) present is time-consuming and often nearly impossible. Finally, microbial lineages likely vary from primarily animal associated to primarily plant associated, either in ecological or evolutionary time. For example, *Lactobacillus* (McFrederick et al. 2012), *Acinetobacter*, and yeasts in the *Starmerella* clade (Lachance et al. 2001, Rosa et al. 2003) each contain species thought to be primarily animal or flower associated, although the tempo or frequency of transitions between host types remains poorly understood.

PROCESSES DRIVING VARIATION IN MICROBIAL ABUNDANCE AND COMPOSITION ON FLOWERS

Variation in floral microbial abundance and composition has been noted at multiple levels: among plant species (Herrera et al. 2009), among tissues of an individual flower (Junker & Keller 2015, Rebolleda Gómez & Ashman 2019), and among individual flowers on a given plant individual or species (Herrera et al. 2009, Vannette & Fukami 2017). Like all ecological communities, floral microbiomes are shaped by multiple processes (**Table 3**). Below, I describe how dispersal (movement of species) and selection (deterministic fitness differences between species) influence community assembly of microbial communities at the scale of individual flowers. Depending on the ecological scale considered, specific mechanisms of dispersal or selection may be more important (**Table 3**). Across longer time periods—including across flowering seasons—additional processes like drift (stochastic change in species abundance) and speciation are probably also important. Although longer-term dynamics of flower microbes have received scant empirical attention and are beyond the scope of this review, they seem a promising area for future research.

Dispersal

Because many flowers are relatively short-lived, dispersal is likely a major determinant of species abundance, composition, and function in flowers. Dispersal from different sources outlined in **Table 2**, including wind, water, plant tissues, or animal visitors, can bring different pools of microbial species. Given the short life span of most individual flowers (Primack 1985) and variability

Table 3 Drivers of variation in microbial abundance and composition in flowers at multiple scales

Scale	Process[a]	Mechanisms involved	Quantifiable variables	Reference(s)
Among plant species	Dispersal	Microbial species pool	Visitation rate and composition of flower visitor community	Herrera et al. 2009, Ushio et al. 2015, Zemenick et al. 2019
	Selection	Resource availability	Nutrient availability	Hypothesized here
	Selection	Environmental filtering	Plant chemistry (volatile and nonvolatile)	Hypothesized here and by Fridman et al. 2012, Junker & Tholl 2013
	Dispersal and selection	Environmental filtering/exposure	Flower morphology	Figueroa et al. 2019, Russell & Ashman 2019
Among flower tissues within a plant species	Selection	Resource availability	Plant nutrient availability	Hypothesized here
	Selection	Environmental filtering	Plant chemistry (volatile and nonvolatile)	Boachon et al. 2019, Huang et al. 2012
	Selection	Environmental filtering	Environmental exposure	Figueroa et al. 2019
	Dispersal	Microbial species pool, dispersal rate	Contact with flower visitors	Rebolleda Gómez & Ashman 2019, Russell et al. 2019
Among flowers on the same plant species or individual	Dispersal	Microbial species pool	Presence and identity of flower visitors	Morris et al. 2020, Ushio et al. 2015, Vannette & Fukami 2017
	Dispersal	Microbial species interactions, historical contingency	Arrival order of flower visitors or microbes	Mittelbach et al. 2016, Peay et al. 2012, Tucker & Fukami 2014, Vannette & Fukami 2014
	Dispersal and selection	Microbial growth, species interactions, changes in flower physiology	Flower age	Morris et al. 2020, Peay et al. 2012
	Dispersal	Metacommunity dynamics, pollinator foraging behavior	Spatial proximity	Belisle et al. 2012, Smessaert et al. 2019
	Dispersal and selection	Direct toxicity to microbes, indirect effects on flower visitor behavior or abundance	Environmental drivers (e.g., nutrient availability, pollutants, agrochemical application)	Bartlewicz et al. 2016, Schaeffer et al. 2017

[a]Processes likely apply at multiple scales but are included where they are predicted to be the most apparent.

in the presence and activity of floral visitors, some flowers may never be visited at all, or not by a particular species of visitor. Contact between flower surfaces and floral visitors can vary with flower morphology or among tissue types, resulting in differential abundance of microbes deposited on flower surfaces (Russell et al. 2019). Variation in which microbial species arrives to a flower first can introduce the possibility of historically contingent assembly patterns (Fukami

2015), with consequences for species richness within individual flowers and the maintenance of microbial diversity across plant populations (Vannette & Fukami 2017).

Selection: Environmental and Floral Traits

Not all microbes that arrive at flowers persist there (Herrera et al. 2010). Microbial establishment and persistence in flowers are restricted by a combination of environmental stressors, host plant traits, and competition among microbial taxa, which result in ecological selection of microbial communities in flowers. Environmental challenges that are similar to those in phyllosphere environments, including UV radiation, desiccation, and patchy nutrient availability, limit microbial colonization of petal or sepal surfaces. As a result, longevity of some microbes on floral surfaces is thought to be short. For the bee pathogen *Crithidia bombi*—one of the few species in which the temporal patterns of survival on flowers have been examined experimentally—the pathogen is infective after being defecated onto petals and sepals, but it survives only hours after deposition (Figueroa et al. 2019). This short life span on flowers has been hypothesized to be due to environmental exposure (Figueroa et al. 2019), but plant traits described below could also reduce microbial survival on floral surfaces.

Floral traits, including chemical, developmental, and morphological phenotypes, can influence microbial growth on flowers (**Table 3**). Although floral traits are typically thought to be adaptations that enhance animal attraction to pollinators, it is likely that some floral traits enhance plant fitness by affecting microbial growth. When considered, it is typically hypothesized that floral traits should decrease microbial growth in flowers (e.g., Adler 2000, González-Teuber & Heil 2009, Rivest & Forrest 2019). However, even if floral traits are adaptations to microbes, not all flower-inhabiting microbes are pathogens (**Table 2**), so it is not clear that flower traits should always evolve to reduce microbial growth. I return to a discussion of flower adaptation to microbes after an overview of the plant traits demonstrated or suspected to influence flower microbial communities.

Many flowers produce abundant volatile organic chemicals, and some of these volatiles affect microbial growth. Flowers of *Arabidopsis thaliana* produce the volatile sesquiterpene (*E*)-β-caryophyllene, which inhibits the growth of *Pseudomonas syringae*, a common leaf-inhabiting bacterium and plant pathogen (Huang et al. 2012), and prevents bacterial damage to the stigma. The volatile (*E*)-β-caryophyllene is commonly produced in floral volatile bouquets (Dobson 2006) and can be induced in other plant tissues following attack by herbivores or pathogens. Other volatiles, including phenylacetonitrile, 2-phenylethyl alcohol, and linalool, can differentially affect bacterial growth rates (Burdon et al. 2018, Hua et al. 2014, Junker et al. 2011). However, in these studies, bacteria isolated from flowers did not always tolerate floral volatiles better than bacteria from leaves did, suggesting that those specific floral volatiles do not necessarily play a large role in structuring floral microbiomes. Nevertheless, some parts of the flower that are particularly sensitive to microbial growth could be defended to a greater extent than others. Flower tissues can vary in volatile composition and emission rate (Boachon et al. 2019, Raguso 2004), and recent evidence suggests complex interplay of volatile emissions and floral morphology that can influence microbial growth. In *Petunia hybrida*, volatile sesquiterpenes are produced before floral anthesis and accumulate on the stigma, effectively fumigating this vulnerable reproductive tissue and altering microbial abundance, diversity, and composition on stigmas (Boachon et al. 2019).

Plant secondary metabolites (nonvolatile chemical compounds not involved in primary metabolism) are also present in flower tissues and may influence microbial growth on flowers (**Table 3**). Floral secondary metabolite composition and concentration differ among plant species and tissues; pollen often contains nearly ten times higher concentrations of secondary metabolites

than nectar (Palmer-Young et al. 2019). Secondary metabolites have been conjectured to have evolved to reduce microbial growth in flowers, among other adaptive explanations (Adler 2000, Rivest & Forrest 2019), but scant evidence to date supports this. In studies that experimentally challenged nectar microbes with secondary compounds, no negative effects on microbial growth were detected (Fridman et al. 2012, Pozo et al. 2012, Vannette & Fukami 2016). However, recent demonstration of the antitrypanosomal activity of callulene, a metabolite isolated from floral nectar of *Calluna vulgaris* (Koch et al. 2019), suggests that other novel metabolites may yet be discovered. Alternatively, it may be that secondary metabolites, in addition to other floral characteristics, reduce flower colonization by environmental or animal-associated microbes (**Table 3**) to a greater extent than floral specialist or phyllosphere microbes, but this hypothesis remains to be tested using in-plant assays.

Additional floral traits have been shown to influence microbial abundance and composition in flower tissues, nectar, and pollen. Within nectar, high osmolarity (Herrera et al. 2010), biochemical conditions maintaining high concentrations of reactive oxygen species (Thornburg et al. 2003), and plant production of small proteins structurally similar to antimicrobial peptides (Schmitt et al. 2018) can limit the growth of some microbes, including generalized fungal pathogens (Carter et al. 1999, Schmitt et al. 2018) and animal-associated microbes (Herrera et al. 2010). Proteins are found in the nectar of many but not all plant species (González-Teuber & Heil 2009, Roy et al. 2017) and can maintain oxidative environments that are antimicrobial or suppress specific activity of fungal enzymes (Naqvi et al. 2005).

Some of the strongest evidence of plant adaptation to reduce microbial growth in flowers comes from the study of plant-induced defenses, including immune responses to floral pathogens (Eastgate 2000). In pepper plants, floral tissue challenged by the pathogen *Xanthomonas campestris* pv. *vesicatoria* upregulated the antimicrobial enzymes chitinase and β-1,3-glucanase faster and to a greater extent than did leaf tissue (O'Garro & Charlemange 1994). Similarly, genomic investigation of apple cultivars that vary in resistance to the pathogen *E. amylovora* have revealed that genes underlying plant immunity and defense pathways are key mediators of variation in plant resistance (Khan et al. 2012). It is unclear if such responses by plants are induced only by coevolved plant pathogens or if other types of floral microbes (**Table 2**) could induce similar changes in plant physiology or defense phenotypes.

Floral traits can affect microbial growth. But have these floral traits been under selection as defenses against microbes? Current evidence is scant. To address this question, studies must examine the variation and heritability of floral traits and the strength of selection, including the relative importance of microbes compared to other selective pressures. In the case of pathogens, the evolution of plant defense is likely, including immune responses described above or other floral traits thought to be under selection by pollinators. For example, larger flowers of the plant *Dianthus silvester* received higher spore deposition of the pathogen *Microbotryum violaceum* (formerly *Ustilago violacea)* compared to smaller flowers of the same species, suggesting that pathogens and pollinators exert opposing selection on floral display (Shykoff et al. 2017). However, for other putatively antimicrobial floral traits, including nectar chemistry and floral volatiles, intraspecific variation has been documented, but heritability and the strength of selection imposed by floral microbes compared to other factors remain unknown (Parachnowitsch et al. 2018).

Selection: Interactions Among Microbes

Interactions among microbes within flowers influence microbial species composition. Microbial species in nectar and on floral stigmas compete for limiting resources. Species that arrive earlier or in higher abundance can preempt these resources, limiting the growth of later-arriving

species (Dhami et al. 2016, Peay et al. 2012, Wilson & Lindow 1994). Microbes can also modify floral environments (e.g., nectar pH or stigmatic surface chemistry) such that later-arriving species are no longer able to establish (Pusey et al. 2011, Vannette & Fukami 2014). Likely as a result of strong competition and niche modification, microbial communities within individual nectar samples are often species-poor and typically numerically dominated by a single yeast or bacterial species (Belisle et al. 2012, Herrera et al. 2010). Because of these interactions, nectar and stigma communities may be prone to historically contingent assembly (Chappell & Fukami 2018, Fukami 2015). For microbial communities on petals or pollen, the role of species interactions in determining composition and relative abundance has received less attention. Here, interactions among microbes could include competition for space or other types of antagonism, including production of antimicrobial compounds (e.g., Kearns & Hale 1995), and may occur on small spatial scales, as seen in phyllosphere communities. Finally, the floral traits outlined above, along with variation in dispersal, can mediate or moderate the outcome of species interactions. For example, nectar sugar or nutrient content can influence the outcome of competition between yeast species (Vannette & Fukami 2014). In contrast, flowers that receive frequent visitation by animals (and as a result, frequent microbial dispersal) may experience weaker species sorting and maintain more microbial species within individual flowers.

MICROBIAL EFFECTS ON FLOWER PHENOTYPE AND ATTRACTIVENESS TO POLLINATORS

After microbes arrive to and successfully colonize flowers, their growth and metabolism can modify floral rewards (nectar and pollen) or phenotypes that attract pollinators. In this section, I review how microbes affect floral rewards and display, and thus plant–pollinator interactions.

How Do Microbes Affect Floral Rewards for Pollinators?

Most flowering plants produce food rewards that attract pollinators, but microbes can alter the quality and quantity of these products directly and indirectly. Floral pathogens can change floral reward phenotypes, including nectar concentration or volume, and alter or eliminate host plant pollen altogether, likely through changes in plant metabolism. Plant species *Silene latifolia* and *Silene dioica* colonized by the anther smut fungus *M. violaceum* produced less nectar, and that nectar had a lower sugar concentration compared to uncolonized plants (Biere & Honders 2006). In contrast, the floral pathogen *Fusarium verticilloides* did not affect nectar volume or sugar concentration of *Moussonia deppeana* flowers, but it did extend flowering time (Lara & Ornelas 2003), increasing nectar production and the time over which nectar rewards were available to pollinators.

Nectar-inhabiting microbes directly alter chemical properties of nectar through their metabolic activity. Nectar yeasts and bacteria typically consume nectar sugars, reducing the sugar concentration (de Vega et al. 2009, Herrera et al. 2008). Nectar microbe metabolism often converts nectar from disaccharide (sucrose) dominated to monosaccharide (glucose and fructose) dominated (Canto et al. 2008, Schaeffer et al. 2015, Vannette & Fukami 2017). Microbial metabolism also affects the concentration and composition of amino acids (Vannette & Fukami 2018) and other chemical constituents, and effects vary depending on microbial identity (Lenaerts et al. 2017, Rering et al. 2018). Although microbial effects on nectar chemistry are often studied in the laboratory using extracted or artificial nectar (e.g., Vannette et al. 2013, Lenaerts et al. 2017), in-flower inoculations can reveal effects not detectable in laboratory assays (Vannette & Fukami 2018). For example, flowers inoculated with *Neokomagataea* (*Gluconobacter*) sp. had lower nectar volume than

controls (perhaps due to accelerated floral senescence) (Vannette & Fukami 2018). Differences between in vivo and in vitro results on nectar sugars and amino acids (Vannette & Fukami 2018) suggest that plant hosts can moderate microbial effects on nectar chemistry. Direct and indirect microbial effects on available calories, nectar taste, or micronutrient composition could plausibly influence pollinator nutrition or foraging (Martínez del Rio 1990). Observations that yeasts in nectar reduce available nectar sugars led to the hypothesis that yeasts compete with pollinators for floral resources (Herrera et al. 2008). In the section titled Consequences of Variation in Floral Microbial Communities, I review evidence for this hypothesis.

Floral microbes could affect pollen quality or quantity, with consequences for plants or animals that consume pollen. Some pathogens have striking effects: The host-sterilizing fungus *M. violaceum* infects host plants in the Caryophyllaceae including the genus *Silene*, where it eliminates female reproductive function of its host and replaces pollen with fungal spores (Alexander & Antonovics 1988). For most microbial groups (**Table 2**), however, evidence for effects on pollen is thin. Most nonpathogenic fungi and bacteria colonize the exterior of pollen after anther dehiscence, so they presumably do not affect pollen production but could affect pollen quality for plants or for pollinators. In the milkweed *Asclepias syriaca*, the presence of nectar-inhabiting microbes decreased the viability of pollen in nectar (Eisikowitch et al. 1990). In this plant species, fertilization occurs via the nectary when pollinia (masses of pollen grains) are deposited into nectar, where they germinate and grow. Nectar yeasts can benefit from pollen nutrients (Pozo & Jacquemyn 2019), but reduced pollen viability could decrease plant reproduction in *A. syriaca*. However, pollen reception via nectaries is relatively rare among plant species, so the extent of nectar microbe effects on male fitness among plant species is unclear. Outside of nectar, filamentous fungi invade pollen, using pollen nutrients to fuel invasion of other plant tissues, including leaves (Fokkema 1971) and reproductive tissues (Huang & Kokko 1985). Pollen consumed by fungi could affect plant reproduction, but this may depend on whether pollen had already been lost from a plant's reproductive pathway (Inouye et al. 1994).

Microbial effects on pollen could also influence pollen-eating animals. Although this topic has received almost no empirical attention, microbes could plausibly affect pollen scent (often used by foraging animals), nutrition, or physiology. After animals gather pollen, it is consumed or, for some insects, stored to feed to offspring. During storage by bees, microbes can spoil nectar and pollen (Batra et al. 1973). Whether soilborne microbes or floral microbes are responsible for spoilage is not understood. Moreover, microbial capacity to access nutrients within pollen could conceivably affect pollen-feeding animals (Roulston & Cane 2000). Pollen is notable in its resistance to degradation. One proposed mechanism of bee pollen digestion is the use of microbial enzymes (Roulston & Cane 2000). Multiple bee-associated bacterial genera contain pectinase genes, whose products cleave bonds in pectin, a complex polysaccharide that makes up the pollen intine (Engel et al. 2012, Vuong & McFrederick 2019); similar genes are also found in the bacterial genera *Acinetobacter* (T.A. Hendry & R.L. Vannette, unpublished data) and *Rosenbergiella* (Laviad-Shitrit et al. 2020), which are commonly found in nectar and on pollen. Determining whether the metabolic activity of floral microbes improves or reduces pollinator ability to access pollen nutrients, however, requires empirical work.

Floral Display

Floral visitors often rely on floral display traits (e.g., floral scent or color) as foraging cues instead of assessing rewards directly. Microbes growing on petals or in nectar (Raguso 2004) are often found to influence floral scent. Bacteria and fungi growing on flowers can both contribute volatile compounds to floral blends or metabolize volatiles produced by plants (thus removing them from

the floral headspace (Farré-Armengol et al. 2016). Flower bacteria can metabolize components of the floral volatile blend detectable to insects, including linalool or 1,2-propanediol (Helletsgruber et al. 2017). In contrast, antimicrobial application reduced terpene emission by nearly 60% in elderberry flowers, suggesting microbes could contribute to terpene emissions (Peñuelas et al. 2014). The nectar yeast *Metschnikowia reukaufii* produces fruity and floral esters (Golonka et al. 2014, Rering et al. 2018). Microbial volatiles produced by nectar specialists (**Table 2**) and phyllosphere microbes can be detected by honey bees and bumble bees (Rering et al. 2018, Schaeffer et al. 2019) and can influence nectar foraging (described in the following section on pollinator foraging). For example, ascomycete yeasts that grow on flowers produce 2-phenylethanol (Rering et al. 2018), a common component of floral blends that is attractive to bumble bees at some doses (Galen et al. 2011) and is antimicrobial at some doses (Hua et al. 2014). Increased emission of the bacterial volatile acetoin has been associated with enhanced attraction of floral visitors (Schaeffer et al. 2019), but this compound has not been validated as an attractant. Actively growing microbes can be isolated from pollen, stigmas, and other floral tissues, but whether these microbes affect volatile composition of entire flowers or tissue-specific volatiles is unclear. Although it is clear that microbes can influence floral scent and volatile-mediated pollinator attraction to flowers in some cases (see examples above), understanding microbial effects on pollinator foraging may be more complex than recognizing innately attractive compounds or competition for floral resources, as I address in the next section.

CONSEQUENCES OF VARIATION IN FLORAL MICROBIAL COMMUNITIES

Microbes likely contribute to the extensive variation observed in floral and nectar characteristics, including nectar volume and composition (Junker et al. 2018, Parachnowitsch et al. 2018). For nectar bacteria and yeasts, abundance is strongly associated with sugar concentration and composition within plant species and can explain up to 65% of the variance in nectar sugar composition (Herrera et al. 2008, Vannette & Fukami 2017). Moreover, as discussed above, microbial identity and abundance in nectar directly influence volatile composition and volatile emission rates (Rering et al. 2018, 2020). Due to variation in microbial abundance and composition, foraging animals are likely to encounter flowers containing few microbes and flowers that have been affected strongly by microbial metabolism, both within and among plant species.

Because microbes generate variability in floral phenotype, microbial cues can covary with floral reward quality or quantity. If yeasts or bacteria degrade floral nectar as proposed by the competition hypothesis above, pollinators should avoid microbe-colonized nectar. In some cases, honey bees, hummingbirds, parasitoid wasps, and bumble bees are deterred by bacteria or fungi in nectar or nectar analogs (Rering et al. 2018, Sobhy et al. 2018, Vannette et al. 2013). However, some nectar microbes promote pollinator visitation instead. Indeed, colonization of flowers by nectar specialist ascomycete yeasts increase floral visitation by bumble bees and parasitoid wasps. The nectar yeast *Metschnikowia reukaufii* increased visitation in multiple studies: by *Bombus* spp. to the plant *Delphinium nuttallianum* in alpine meadows of the Rocky Mountains in the United States (Schaeffer & Irwin 2014), by *Bombus terrestris* and *Bombus pratorum* to the early-blooming *Helleborus foetidus* in woodlands in Spain (Herrera et al. 2013), and by *Bombus friseanus* to *Clematis akebioides* in southwestern China (Yang et al. 2019). In each case, free-flying bumble bees preferred microbe-colonized nectar to uncolonized nectar of the same plant species. These ascomycete yeasts decrease available sugars, by nearly 60% in some cases (Herrera et al. 2008), suggesting that bumble bees are foraging for lower-quality nectar—the opposite of the direction proposed by the competition hypothesis. Moreover, when bumble bees or parasitoid wasps are fed yeast-conditioned nectar in no-choice assays, negative effects on individual growth or survival

are rarely documented, even under resource-limited conditions (Pozo et al. 2020, Schaeffer et al. 2017, Sobhy et al. 2018). On the contrary, floral yeasts present in the diet of the bumble bee *B. terrestris* often enhanced bee colony size and decreased mortality (Pozo et al. 2020). Thus, despite shared use of nectar resources by nectar microbes, bumble bees, and other floral visitors, evidence for direct competition or clear antagonism remains scant.

An alternative, the honest cue hypothesis, proposes that nectar yeast metabolites act as informative (honest) cues of nectar rewards (Pozo et al. 2009, Raguso 2004, Schaeffer & Irwin 2014). This hypothesis proposes that yeast metabolism may more accurately represent reward presence or quality than do floral signals such as plant-produced floral volatiles. But is this hypothesis supported? In a laboratory experiment, naive and trained workers of *B. impatiens* initially used yeast volatiles for foraging but reduced preference for yeast-colonized nectar over foraging bouts, presumably because visual cues were more informative of the presence of nectar (Schaeffer et al. 2017). This and other experiments have shown that bumble bees' preferences for yeast volatiles are not fixed; bees can prefer bacterial volatiles to yeast volatiles (Schaeffer et al. 2019) or learn to associate either bacterial or yeast cues with the presence of a reward (Russell & Ashman 2019). In addition, yeast volatile emission rate varies with nectar chemistry, suggesting that the amount of volatile produced may change with nectar quality. In solutions of varying sugar concentration (between 5% and 45% sugars), yeast volatile emission rate increased with sugar concentration despite little change in yeast cell density among nectar types (Rering et al. 2020), suggesting that volatile emission rate could cue the presence of higher sugar nectar rewards. However, support for the honest cue hypothesis does not rule out other explanations for pollinator attraction to yeasts. Yeasts could also contribute to nutrition, diet detoxification, pathogen protection, or additional mechanisms specific to the animals' ecology (Pozo et al. 2020). The effects of other floral microbes on floral visitor foraging have received less attention.

DO PLANTS BENEFIT FROM FLORAL MICROBES?

Floral microbes rarely benefit plants. Indeed, flower pathogens and some bacterial nectar microbes can reduce plant fitness, either directly or by decreasing pollinator visitation (Vannette et al. 2013). Furthermore, although nectar yeasts can increase pollinator visitation to flowers (summarized above), increased visitation does not always benefit plant fitness. Nectar yeasts can increase male fitness of colonized plants or pollen receipt by flowers (Schaeffer & Irwin 2014, Yang et al. 2019). However, yeast presence can also reduce seed set of yeast-colonized plants despite increased pollinator attraction (Herrera et al. 2013), perhaps due to reduced pollen quality from self-pollen transfer rather than outcrossed pollen. Although limited data are available, it may be that plants benefit from microbe-enhanced visitation by pollinators only under a limited set of conditions.

Even in deceptive pollination systems, where plant reproduction depends on attracting vectors to microbial volatiles, plants mimic microbial scents associated with carrion or decomposition to attract flies or beetles instead of relying on microbes to produce those volatiles (Urru et al. 2011). However, a few documented cases suggest plant adaptation to promote microbial growth in flowers. In one striking example, the bertam palm (*Eugeissona tristis*) from West Malaysia bears long-lived (over 4 months in some cases), robust flowers that contain abundant yeast populations. Yeast fermentation, which occurs in central chambers of the robust flower, generates floral nectar containing nearly 4% ethanol. The palm's mammal pollinators—tree shrews, squirrels, and nocturnal murids—are adapted to chronic alcohol consumption (Wiens et al. 2008). Although no conclusive evidence demonstrates that palms depend on microbes for pollinator attraction, this example suggests that plants could exhibit morphological adaptations that promote microbial growth in flowers and associate with pollinators adapted to consuming fermented nectar. Extensive microbial growth can also be found in plant clades that rely on bird or mammal pollination

(de Vega et al. 2009, Rourke & Wiens 1977). If floral microbes enhance plant fitness in these plant species, they could be useful models for examining the potential for plant adaptations to enhance microbial growth in flowers.

HOW DO MICROBES GET OUT OF FLOWERS?

Flowers are generally short-lived (Primack 1985), so flower microbes must colonize alternative habitats or new flowers to maintain population growth. Some flower microbes invade plant vascular tissue (**Table 2**), infect seeds, or metabolize decaying flower tissue after abscission (Lachance et al. 2001). For microbes that can colonize plant or animal tissues, dispersal offers access to uncolonized hosts (plants or animals) and enables sexual reproduction (Roy 1993). For flower specialists, transport to other flowers is essential, and flowers offer excellent opportunities for microbial dispersal: A potential vector arrives on average every 4 min, or for infrequently visited plant species, up to every 14 h (Herrera 2020). The density of insects is also higher on flowers than on surrounding leaf tissue (Wardhaugh et al. 2012). Likely because of the frequency and diversity of potential vectors at flowers, diverse microbes (**Table 2**) take advantage of dispersal opportunities there.

Some plant-associated microbes make their own dispersal opportunities by manipulating plants to produce structures that physically and chemically resemble flowers (Roy 1993). The floral pathogen *Monilinia vaccinii-corymbosi* induces blueberry host plants to form spore-containing pseudoflowers on leaves and produce floral volatiles that attract bees (McArt et al. 2016). Even rhizosphere plant bacteria may take advantage of dispersal opportunities at flowers. A strain of *Streptomyces* bacteria, frequently found associated with strawberry roots, is able to translocate through plant vascular tissues to flowers (Kim et al. 2019), where it is dispersed by pollinator visitation. Growth of *Streptomyces* in flowers reduces growth of the pathogenic fungus *Botrytis* and, in honey bees, reduces mortality from a bacterial pathogen. This study suggests a complex, dispersal-mediated mutualism among bees, plants, and bacteria (Kim et al. 2019).

As discussed in the previous section, microbes can increase or decrease attractiveness of flowers (Vannette et al. 2013), but whether these changes influence microbial dispersal probability is often presumed rather than quantified. For nectar yeasts, bumble bees preferentially visit yeast-colonized flowers (Herrera et al. 2013, Yang et al. 2019), and yeasts are found in visited but not in unvisited flowers. It is also plausible that microbes could produce chemical compounds that attract specific vector species. Microbial specialization to particular vector animal species that exhibit host plant fidelity could allow for microbial specialization to specific hosts and their chemical traits. Although there are many documented examples of flower visitor species vectoring specific microbial species or strains to flowers (Lachance et al. 2001, Morris et al. 2020, Ushio et al. 2015) (see the section titled Dispersal), no evidence yet demonstrates that microbial species attract specific vector species to flowers and simultaneously rely on these animals for dispersal.

Besides attractiveness, microbes may affect other floral traits to increase dispersal probability. The fungal pathogen *Fusarium moniliforme* increases floral longevity of its host plant, enhancing visitation by birds and dispersal of fungal spores (Lara & Ornelas 2003). Many plant phyllosphere yeasts and bacteria can produce plant hormones, including indole-3-acetic acid, which can influence floral development or longevity (Roy et al. 2017). However, whether the ability to affect floral longevity is present or widespread among microbial species is unknown.

FUTURE DIRECTIONS

When and to What Extent Do Microbes Drive the Evolution of Floral Traits?

Pollinators are still regarded as the main drivers of the evolution of plant traits, but this review and previous papers have outlined how floral traits can also affect microbial growth and hypothesized

a role for microbes in the evolution of floral traits (Adler 2000, McArt et al. 2014, Parachnowitsch et al. 2018, Rebolleda-Gómez et al. 2019, Rivest & Forrest 2019). Scant progress has been made in answering this question. To determine the role of microbes in the evolution of floral traits, the answers to two questions are necessary. First, does heritable variation exist in floral traits that influence microbial growth? Second, what is the strength (and direction) of selection by floral microbes on plant traits, particularly compared to other known drivers of floral traits, including pollinators and floral antagonists? Assuming that microbes do influence the evolution of floral traits, are the targets of selection the same traits as those under selection by pollinators, such that floral microbes could limit plant adaptation to pollinators, or are the traits largely distinct? In addition, which groups of microbes (**Table 2**) most strongly influence the evolution of floral traits?

Which Components of the Floral Microbiome Are Most or Least Variable?

I have argued that variability in microbial presence, abundance, and composition is a defining feature of the floral microbiome. Abundant evidence supports this assertion for microbes in nectar and on stigmas, and to some extent on pollen and petals (Brysch-Herzberg 2004, Herrera et al. 2009, Pusey et al. 2009, Shade et al. 2013, Vannette & Fukami 2017). However, whether particular floral tissues, plant species, or geographic regions are more variable or consistent than others (Rebolleda-Gómez et al. 2019) is not yet clear. Future work should strive to characterize microbial abundance and composition of microbial species and strains across individual flowers and tissues in addition to among plant species or communities to better understand where variation in the microbiome exists. Despite the technical challenges in quantifying floral microbes at small scales (**Table 1**), a better understanding of the scale of variability will inform the biology of nectar microbes, the types of interactions important in flowers, and how best to manage floral microbiomes and their effects on plants and floral visitors.

Under What, If Any, Conditions Are Flower Microbes Beneficial for Plants?

Historically, work on floral microbes has focused on microbes pathogenic or detrimental to plants or pollinators. It is quite conceivable, though, that some floral microbes can be beneficial under specific conditions. For example, a better understanding of when floral yeasts could enhance pollination, and specifically male fitness, might be useful in agricultural or restoration settings. It is also plausible that floral microbial communities could provide protection against floral pathogens, as has been demonstrated for phyllosphere microbial communities (Finkel et al. 2017). Microbial biocontrol agents of floral pathogens *E. amylovora* and *Monilinia* spp. have been isolated from leaf or floral surfaces and are used in agriculture (Lindow & Suslow 2003, Pusey 1999, Pusey et al. 2009). However, it remains unknown if floral microbial communities offer natural pathogen control and which biotic or abiotic conditions affect microbiome-mediated pathogen protection. Future work could examine if particular characteristics of floral microbiomes (e.g., their diversity, abundance, or species composition) enhance plant protection against floral pathogens.

Under What, If Any, Conditions Are Flower Microbes Beneficial for Pollinators and Other Floral Visitors?

The effects of floral microbes on floral visitors, outside of direct pathogenic effects, remain poorly understood. Nectar yeasts may enhance foraging efficiency and could enhance queen overwintering and colony growth (Pozo et al. 2020), although this may depend on whether yeasts are already

present in the bumble bee colony (Schaeffer et al. 2017). Nectar-dwelling bacteria can also affect longevity of adult parasitoid wasps (Lenaerts et al. 2017). However, in both cases the mechanisms are poorly understood and may be context dependent. Flower microbes could affect other flower-visiting animals, including those in egg or larval stages. For example, some solitary bee species provision their eggs with nectar and pollen in chambers called brood cells. Provisions contain microbes from nectar and pollen (Voulgari-Kokota et al. 2018), which grow on these stored resources. It has been hypothesized that microbes, including those acquired at flowers, may ferment these stored protein and carbohydrate sources, potentially preserving provisions by suppressing the growth of fungi or bacteria that would otherwise spoil stored food resources (Batra et al. 1973, Danforth et al. 2019, Voulgari-Kokota et al. 2020). Recent evidence also suggests that microbes that grow on stored provisions make up part—in some cases, a major component—of the bee diet (Steffan et al. 2019). Despite these intriguing results, effects of flower microbes on the ecology and fitness of floral visitors in realistic conditions remain to be examined, and the mechanisms by which microbes influence animal survival and growth remain to be described. Given the diversity of animal lineages that visit flowers—hoverflies, thrips, solitary and social bees and other Hymenoptera, beetles, butterflies, birds, and lacewings, to name a few—the mechanisms and magnitude of effects of floral microbes on foraging and fitness are also likely to vary.

CONCLUSIONS

Microbial inhabitants of flowers are common third-party players in pollination mutualisms that influence both plants and pollinators on ecological and likely evolutionary timescales. As I have described, however, microbial species vary in their ecologies and effects on pollination. A better understanding of the drivers of microbial species composition on both small scales (e.g., on pollen) and large scales (e.g., across flowering seasons for individual plant species, across species, or across habitat types) will reveal the ecological strategies of microbes, the microbial landscape in which flowers and pollinators interact, and the processes that shape complex multispecies interactions among plants, microbes, and animals.

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