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Floral traits affecting the transmission of beneficial and pathogenic pollinator-associated microbes

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Flowers provide resources for pollinators, and can also be transmission venues for beneficial or pathogenic pollinator-associated microbes. Floral traits could mediate transmission similarly for beneficial and pathogenic microbes, although some beneficial microbes can grow in flowers while pathogenic microbes may only survive until acquired by a new host. In spite of conceptual similarities, research on beneficial and pathogenic pollinator-associated microbes has progressed mostly independently. Recent advances demonstrate that floral traits are associated with transmission of beneficial and pathogenic microbes, with consequences for pollinator populations and communities. However, there is a near-absence of experimental manipulations of floral traits to determine causal effects on transmission, and a need to understand how floral, microbe and host traits interact to mediate transmission.

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Introduction

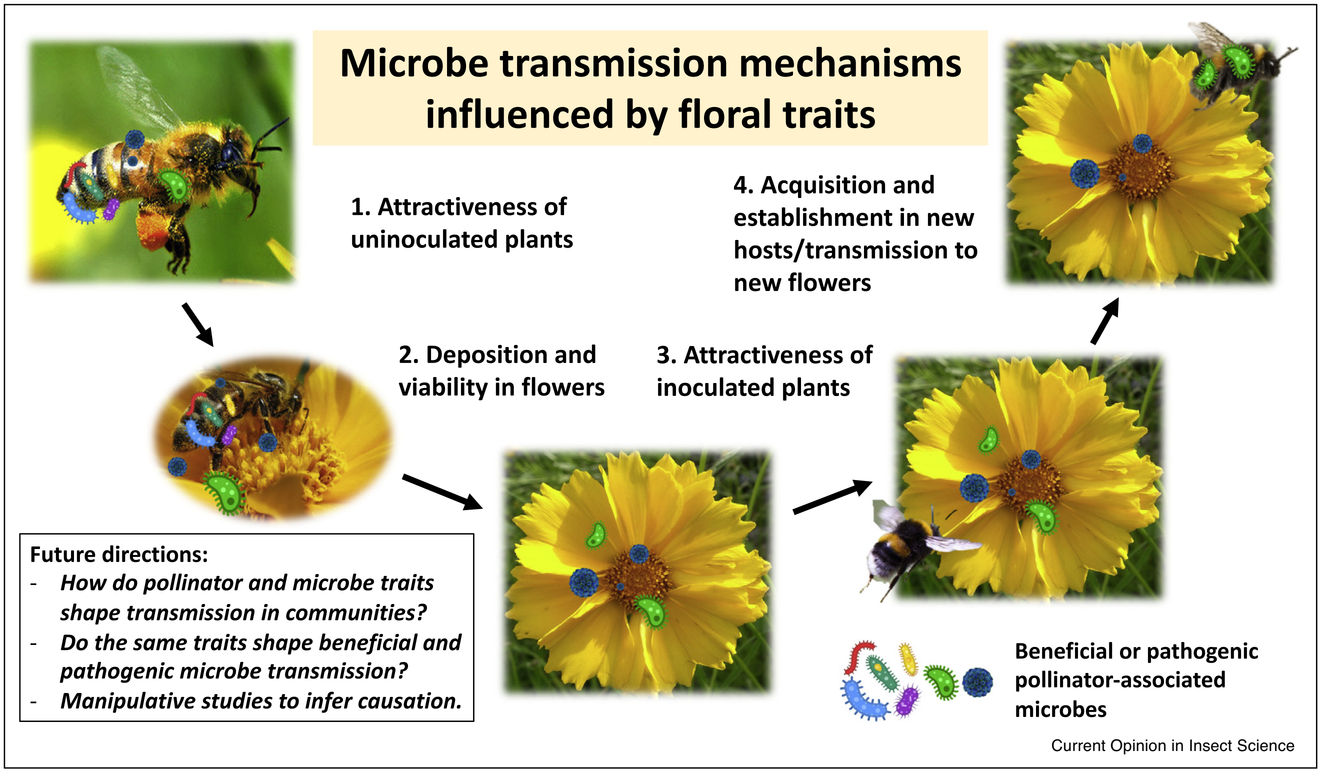
By providing pollen and nectar in attractive displays, flowers serve as 'nature's rest stop' in all its connotations: a source of resources but also a receptacle for waste and a way station for passing travelers that mix and then disperse. Flowers can have insect densities 10 000 times greater than surrounding foliage [1] and so may be sites where microbes are transmitted between visitors and other flowers. A 2014 review assessed the role of floral traits in transmission of pathogens to plants and pollinators via flowers [2]; while there was a substantial literature for plant pathogens, at that time only a single study had experimentally demonstrated transmission of pollinator pathogens at flowers. Since then, there has been growing research on pollinator pathogens, alongside a parallel but largely separate literature understanding how floral traits affect transmission of microbes beneficial to pollinators.

Here, we review recent studies of transmission of both beneficial and pathogenic pollinator-associated microbes. We define 'beneficial' and 'pathogenic' microbes in terms of their effect on pollinators; 'pathogenic' microbes have a detrimental impact on at least some pollinators, while 'beneficial' microbes have a positive impact on at least some pollinators, although the ecology of many microbes is poorly understood and effects on pollinators could be context-dependent. Further, we structure this new review by the four mechanisms proposed in the 2014 review [2]: (1) Floral attractiveness of uninoculated plants, (2) Microbe deposition and viability in flowers, (3) Floral attractiveness of inoculated plants, and (4) Pathogen acquisition and establishment in hosts upon visiting inoculated flowers (Figure 1). With this organizational structure, our goal is to highlight similarities and differences in the role of floral traits on transmission of microbes that may be beneficial, commensal or detrimental to pollinators, with the goal of a more mechanistic understanding of the role of floral traits in these interactions. Although many microbes that affect plants can be transmitted via flowers [2], this is outside the scope of our review. We end by highlighting gaps in knowledge and identifying future key areas of interest.

Traits influencing transmission of beneficial microbes

Many microbes on flowers cause no detectable harm to plants or pollinators, and may in some cases benefit them. Yeasts and bacteria are common inhabitants of flowers and are often more abundant and frequently isolated after pollinators have visited a flower compared to unvisited flowers [e.g. Refs. 3,4], suggesting that pollinators are major transmitters. Flowers and pollinator bodies are distinct environments that differ in nutrient composition, environmental conditions, and longevity, and so it is no surprise that microbial species appear to specialize on these distinct habitats. For example, the Ascomycete

Figure 1



Floral traits can influence beneficial or pathogenic pollinator-associated microbe transmission in four ways. Trait can influence 1) the attractiveness of uninoculated plants to pollinators, 2) deposition and viability in flowers, 3) attractiveness of inoculated plants, and 4) acquisition and establishment in new hosts/transmission to new flowers. Each of these four mechanisms is highlighted in the text, along with a brief discussion of promising future directions. Beneficial and pathogenic pollinator-associated microbes are represented by the caricatures of different colors.

yeasts *Metschnikowia reukaufii* and *Metschnikowia gruessi* attain high density in flowers but are detected in low numbers on or in bumble bee bodies. In contrast, Ascomycete yeasts in the *Starmerella* clade and the genus *Debaryomyces* can be detected in low numbers on flowers, but attain high densities in bumble bee gastrointestinal tracts and honeypots [5].

Some species of fungi and bacteria found on flowers can benefit pollinators. Consumption of nectar containing yeasts can increase colony growth of *Bombus terrestris* and may protect against pathogens [6]. Bumble bees can use microbial volatiles as foraging cues [7•], which may increase foraging efficiency or resource acquisition. Microbes in nectar and pollen can colonize solitary bee provisions [8], where they may aid in preserving sugar and protein, or serve as food for developing larvae [9,10]. Many other insects deposit microbes on floral surfaces and/or consume microbes at flowers, and microbes could benefit flower-feeding insects in diverse ways [11].

Despite the central importance of flower–pollinator interactions in the transmission of beneficial microbes, surprisingly little is known about how floral traits affect transmission. Our review focuses on nectar fungi and bacteria, with recognition that research on other microbial symbionts awaits detailed investigation [12].

(1) Floral attractiveness of uninoculated plants

Because many nectar microbes require pollinator visitation for transmission, presumably any floral traits that increase floral visitation (overall or by taxa that carry specific microbes) will increase transmission. For example, nectar yeast frequency and abundance are often positively correlated with pollinator visitation. In addition, yeast abundance in nectar was positively associated with the proportion of floral visits by bumble bees, but negatively correlated with visits by solitary bees [13], suggesting that floral traits associated with particular pollinator groups may affect transmission [14]. However, research is needed that links intraspecific and interspecific trait variation to transmission [akin to Ref. 15••]. One exemplar study found higher nectar microbial abundance in male compared to female flowers of *Eurya emarginata*, but the role of pollinator transmission versus filtering by the nectar environment or resource availability between flower types was not resolved [16•].

(2) Microbe deposition and viability in flowers

Microbes in nectar are often a subset of those found on and in pollinator bodies, suggesting strong filtering by nectar, likely favoring species that can withstand the high sugar environment [17] or grow quickly [18]. Research is accumulating on how nectar traits affect microbe viability and growth, although it is sometimes challenging to separate plant species identity versus trait-based effects, and strains of the same microbial species can vary widely in relative growth rates in the same nectar sources [19]. However, some common patterns have emerged. For example, high sugar concentration can inhibit microbial growth, and nectar secondary compounds, once thought to reduce microbial growth in nectar, drive concentration-dependent and compound-specific effects that often don't inhibit growth at natural concentrations [18,20]. Survival and growth of microbes in nectar also depends on their interactions. For example, nectar bacteria and yeast experience strong priority effects, with whoever arrives first or has higher initial abundance suppressing the other [21,22]. Mechanisms associated with priority effects are likely related to microbial growth rate [18] and subsequent effects of microbes on nectar traits, such as pH and amino acids [22,23]. Floral traits that affect microbial viability and growth in turn affect microbe acquisition. In artificial nectar arrays, transmission can depend strongly on microbial density in flowers [24]. These studies lead to the prediction that floral traits that promote microbial growth will also promote microbial transmission among pollinators (Figure 1).

(3) Floral attractiveness of inoculated plants

Many nectar microbes affect floral attractiveness to pollinators. For example, both artificial and natural flowers inoculated with the nectar yeast *M. reukaufii* receive increased bumble bee pollinator visitation relative to uninoculated flowers [25,26], whereas nectar and floral surfaces colonized by bacteria (such as *Neokomagataea* sp. formerly *Gluconobacter* sp.) can reduce visitation by hummingbirds, bumble bees, and honey bees [27–29]. Although mechanisms may vary, strong evidence suggests that pollinator attraction of yeast-inoculated flowers is driven by associative learning of yeast-derived volatiles with floral rewards [28], as well as gustatory responses once pollinators start feeding [30••,31••].

(4) Microbial acquisition by pollinators and transmission to new nectar sources

Little is known about how floral traits affect the likelihood that pollinators will acquire microbes from nectar sources, but some exemplar behavioral work suggests a role. Bees acquire approximately 1% of the microbes on flowers, with less acquired from nectaring than collecting pollen [32]. Thus, floral traits that increase time spent accessing rewards or proportion of the pollinator's body contacting contaminated nectar or surfaces should increase acquisition. Three-way interactions among flower, microbe and pollinator traits are likely important in the transmission process, and assessing how microbe traits interact with floral and pollinator traits to affect dispersal may yield unique insights [11].

Traits influencing transmission of pathogenic microbes

Floral traits could mediate the transmission of pathogenic microbes similarly to beneficial microbes, but there may also be key differences. Some beneficial microbes can establish and grow in and on flowers, and floral traits may shape their growth. By contrast, pollinator pathogens typically cannot grow on or in flowers (although this is rarely examined) and so floral traits may affect pathogen survival and transmission, but not growth. We describe how floral traits could affect pathogen transmission using the same mechanistic structure as for beneficial microbes to facilitate comparisons.

(1) Floral attractiveness of uninoculated plants

Pathogen deposition on flowers can differ by plant species, which could be due to attraction or how pollinators interact with flowers. The bumble bee pathogens *Nosema bombi*, *Crithidia bombi* and *Apicystis bombi* had more deposition onto bell-shaped (*Campanula cochleariifolia*) than flat (*Viola tricolor*) flowers, but deposition of *Nosema apis* and *Nosema ceranae* honey bee pathogens did not differ between plant species [33]. Two honey bee viruses had uneven deposition onto flowers of three legume species, and deposition differed when plant species were presented alone versus in mixtures [34••]. Flowers with the longest but also fewest honey bee visits had the highest virus loads, suggesting that floral traits affect deposition by altering bee visitation and behavior. *Bombus impatiens* infected with *C. bombi* had variable likelihoods of depositing feces (containing infective cells) on different floral parts in three plant species [35•], suggesting that floral morphology affects deposition, and *B. impatiens* were also more likely to defecate on a large composite flower than flowers of seven other species [36]. Bumble bees infected with *C. bombi* spent more time in flowers with high-iridoid glycoside nectar than uninfected bees and were more likely to return to other high-iridoid flowers [37], both of which could affect pathogen deposition.

(2) Pathogen deposition and viability in flowers

In a comprehensive study, Figueroa *et al.* [38•] found at least one bee pathogen (including neogregarines, trypanosomatids, *N. ceranae* and *N. bombi*) in flowers from 75% of 13 plant species from multiple field sites, with pathogen prevalence differing widely between plant species. These differences could be due to differential visitation or acquisition and viability between plant species, but the traits responsible are unknown. Once deposited on flowers, pathogens may contact nectar [but see Ref. 39] and as noted for beneficial microbes, the nectar environment may be challenging for pathogen survival. Exposure to increasing sugar concentrations before consumption reduced *C. bombi* infection likelihood and intensity in bumble bees [40]. *C. bombi* exposure to the nectar iridoid glycoside aucubin before consumption also reduced subsequent bumble bee infection [41], but exposure to several other nectar secondary compounds did not [41,42]. Location on flower parts can also affect viability; *C. bombi* survival was lower on exposed bracts than inside flowers and in sun compared to shade [35•].

(3) Floral attractiveness of inoculated plants

Although *B. terrestris* avoided foraging on flowers inoculated with *C. bombi* [43], we are aware of no studies assessing whether floral traits mediate bee responses to inoculated plants. Given the strong evidence that beneficial microbes affect floral attractiveness, this mechanism warrants further investigation for pathogens.

(4) Pathogen acquisition and establishment in hosts upon visiting inoculated flowers

Over 25 years ago, a seminal paper demonstrated that *B. terrestris* and *Bombus lucorum* became infected with *C. bombi* after foraging on flowers visited by infected bees [44]. The odds of acquiring infection differed between two plant species, and also in inflorescences manipulated to change floral architecture. This study incorporates the role of floral traits on both deposition by infected hosts and acquisition/establishment in new hosts. To our knowledge this is the only paper that has manipulated any floral trait to assess consequences for pathogen transmission in pollinators.

That said, several nectar secondary compounds consumed after pathogen acquisition can reduce *C. bombi* and *N. ceranae* infections *in vivo* [reviewed in Refs. 45–47], with one study discovering a mechanism; callumene from *Calluna vulgaris* nectar removed the *C. bombi* flagellum, preventing attachment to gut walls [48•]. However, adding the nectar compound thymol to flowers along with *C. bombi* did not affect pathogen establishment in foraging bumble bees [49].

Flowers, by acting as deposition and acquisition venues, may also be important in pathogen transmission between host species. An RNA virus was transmitted between honey and bumble bees co-foraging on the same flowers, but this study did not eliminate the possibility of transmission via other surfaces such as cage walls [50]; a more recent study found that honey bees deposited viruses on flowers, but viruses were not subsequently acquired by bumble bees [34••]. However, stingless bees (*Tetragonula hockingsi*) became infected by *N. ceranae* that was previously deposited on flowers by *Apis mellifera* [51], conclusively demonstrating the role of flowers in transmission between bee species. Vectoring of bumble bee pathogens by honey bees differed on two plant species, but vectoring of honey bee pathogens by bumble bees did not differ with plant species [33]. Furthermore, parasites can play a role in vectoring pathogens; *Varroa* mites, which transmit deformed wing virus to honey bees, were slowest to infest honey bees foraging at *Echinacea* flowers compared to flowers of two other plant species, suggesting a role of floral shape [52].

Finally, one observational study attempted to isolate floral traits shaping pathogen acquisition. *C. bombi* was added to flowers of 14 plant species, after which individual *B. impatiens* foraged. There was a fourfold difference across plant species in pathogen acquisition and infection intensity [15••]. However, floral size and shape, number of open flowers, nectar production, and inflorescence height did not explain interspecific variation in transmission; the only trait that correlated with pathogen acquisition was the total number of reproductive structures per inflorescence.

Traits influencing microbe spread in plant–pollinator communities

The studies above indicate that floral traits can influence transmission at individual flowers, but how these individual interactions shape microbe spread in communities is just beginning to be examined. Susceptible-infectious-susceptible (SIS) models for plant–pollinator networks were recently developed with continuous trait distributions, finding that disease spread was impacted the most by selective pollinators, universally attractive flowers, and cospecialized plant–pollinator pairs [53••]. Although this theory was developed for pathogenic microbes, it can also be applied to beneficial microbes.

Two recent studies show that prevalence of pathogenic microbes on flowers varies among plant species, and changes in the bee:flower ratio in communities can influence the likelihood of transmission at flowers. Graystock *et al.* [54••] screened >5000 bees and flowers in old-field communities and detected bee pathogens (*N. bombi*, *N. ceranae*, *C. bombi*, *Crithidia expoeki*, or *Apicystis* spp.) in 42% of bee species (12.2% individual bees) and 70% of flower species (8.7% individual flowers). Prevalence varied by more than 80% among well-sampled flower species. In addition, prevalence on flowers was lowest late in the season when the bee:flower ratio was lowest, suggesting reduced risk of transmission via dilution. Supporting the potential importance of dilution, an experiment manipulating *B. terrestris* density in replicated plant communities found that when the bee:flower ratio was low, slow bee paralysis virus (SBPV) was transmitted less efficiently [55•]. This pattern was not observed for *C. bombi*, indicating parasite-specific responses to bee/flower density.

New work also suggests that visitation by particular pollinator species to the flowers of particular plant species can potentially play a disproportionate role in transmission. *A. mellifera* visitation rates to nectar-rich knapweed (*Centaurea* spp.) were greater than visitation rates of the rest of the pollinator community combined to this plant species [56], and honey bees and knapweed were both among the highest-prevalence bee and flower species, respectively, for multiple pollinator pathogens [54••].

Finally, particular plant species can be associated with reduced pathogens in pollinators, and community-level changes in floral traits can shape disease intensity. Sunflower pollen dramatically reduced *C. bombi* infection in bumble bees, and farms with more sunflower had bees with lower infection intensity [57]. Bumble bees at Belgian sites invaded by *Impatiens glandulifera* had lower prevalence of infection with *Apicystis* but not other pathogens; the authors hypothesized that pollen polyphenols could decrease infection [58]. Wildflower fields increased prevalence of several bee pathogens as well as bee abundance in some landscape contexts [59•]. Finally, Adler *et al.* [60•] created replicated communities using plant species that had resulted in high or low *C. bombi* infection in previous flower foraging assays [15••]. Colonies foraging in tents with high-infection plant species had on average twice the infection intensity compared to colonies with low-infection plant species, indicating the importance of plant communities for infection dynamics [60•]. Identifying the floral traits mediating such interactions would provide an invaluable tool for choosing species to include in habitat restoration.

Future directions

Although there are many gaps in our understanding of how floral traits affect microbe transmission, here we briefly highlight ideas that may be particularly productive.

Assessing the effect of floral traits on multiple microbes

Various pathogens may interact with hosts and flowers differently, and some have been more strongly associated with pollinator decline than others. Which floral traits are most important for the transmission of the most detrimental pathogens or the most beneficial microbes, which are also critical for pollinator health? Currently, observational studies suggest patterns but there are almost no experimental manipulations of floral traits to determine causal relationships. Such information is essential to provide a general framework that could guide choices of plants for pollinator habitats, as well as understand trait-mediated host–parasite dynamics.

The role of floral traits in a community context

Do beneficial microbes and pathogens frequently co-occur in particular plant species? Are plant species with more similar floral traits more likely to share microbial communities, and are such correlations structured by floral traits and/or pollinator visitation? Are some traits generally anti-microbial, reducing both pathogen and beneficial microbe growth? Similarly, are pollinator species with more similar traits or behaviors more likely to share pathogens or transmit beneficial microbes? Research on beneficial and pathogenic microbe transmission has grown almost independently, but combining them could yield important insights for bee health.

Interactions among flower, host, and microbe traits

Our focus has been on how floral traits affect microbe transmission, but the transmission process will also depend on traits and behaviors of pollinators and microbes. For example, the morphology or other properties of particular microbes may make them more conducive to dispersal by particular pollinators [11], or among particular flower morphologies. In addition, transmission could occur via biotic or abiotic vectors, and the biology of the microbe may affect the efficacy of each of these routes. If this is the case, studying the traits of flowers alone may misrepresent the drivers of microbial transmission. Increased study of pollinator and microbial species using a trait-based approach may help identify the 3-way community trait space most conducive or susceptible to microbial transmission and whether such traits can be generalized across interactions.

Credit author statement

Rachel Vannette and **Rebecca Irwin**: wrote the 'Traits influencing transmission of beneficial microbes' section. **Scott McArt**: wrote the 'Traits influencing microbe spread in plant–pollinator communities' section and drafted the figure. **Lynn Adler**: wrote the abstract, introduction and 'Traits influencing transmission of pathogenic microbes' section. **All authors**: co-wrote the Highlights, Future Directions, and provided feedback on all sections and the figure.

Conflict of interest statement

Nothing declared.

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