Viewpoint

Gazing into the anthosphere: considering how microbes influence floral evolution

Summary

The flower is the hallmark of angiosperms and its evolution is key to their diversification. As knowledge of ecological interactions between flowers and their microbial communities (the anthosphere) expands, it becomes increasingly important to consider the evolutionary impacts of these associations and their potential eco-evolutionary dynamics. In this viewpoint we synthesize current knowledge of the anthosphere within a multilevel selection framework and illustrate the potential for the extended floral phenotype (the phenotype expressed from the genes of the plant and its associated flower microbes) to evolve. We argue that flower microbes are an important, but understudied, axis of variation that shape floral trait evolution and angiosperm reproductive ecology. We highlight knowledge gaps and discuss approaches that are critical for gaining a deeper understanding of the role microbes play in mediating plant reproduction, ecology, and evolution.

Introduction

Flowers function for sexual reproduction and seed and fruit development, and it is acknowledged that their interactions with pollinators and seed dispersers lead to the diversification of angiosperms (Magallón *et al.*, 2018). As a complex, nutrient-rich structure, the flower is also a unique environment for microbial communities: it consists of multiple niches differing in morphology, chemical composition and longevity. The function of these niches changes over the course of the flower's life (Junker *et al.*, 2011; Aleklett *et al.*, 2014; Wei & Ashman, 2018).

Microbial communities of the flower (anthosphere) are distinct from those of the leaf (phyllosphere), root (rhizosphere), soil, and pollinator, although all may share many members (Junker *et al.*, 2011; Allard *et al.*, 2018; Wei & Ashman, 2018; Rebolleda-Gómez & Ashman, 2019). Floral microbes (bacteria and fungi) can be transferred horizontally among flowers by pollinators (Russell *et al.*, 2019), wind or rain, or vertically between plant and seed (reviewed in Nelson, 2017). Floral microbes can be endophytic or epiphytic. Endophytic microbes reside within floral tissues, such as carpels and seeds, whereas epiphytic microbes, the primary focus of this viewpoint, are found in abundance (9×10^4 to 1.5×10^6

microbial cells per flower; Russell & Ashman, 2019) on the surface of all floral structures (petals, sepals, carpels, stamens), and within nectar (up to 1×10^7 microbial cells per microliter of nectar; Rering *et al.*, 2018). These floral microbes may consume volatiles and other exudates (mostly small sugars and amino acids), similar to foliar bacteria (Mercier & Lindow, 2000; Pusey *et al.*, 2008), as well as floral waxes (Lachance *et al.*, 2001). Bacteria and yeast that inhabit nectar feed on sugars, amino acids and proteins therein (Pozo *et al.*, 2012; Vannette *et al.*, 2013; Vannette & Fukami, 2016). Floral microbes (fungi, bacteria archaea, protists and viruses) might range from mutualistic to pathogenic, in many cases their effects on plant fitness are likely to depend on the ecological context. Studies in flowers, however, have focused primarily on fungi and bacteria, and although dynamics of other microbes (archaea, protists and viruses) remain largely unknown, work in the phyllosphere suggests that these dynamics might also be important to understand ecology and evolution in the anthosphere (Koskella *et al.*, 2011).

While we are just beginning to gain a broad perspective on the abundance and diversity of microbes within the anthosphere, some patterns are starting to emerge. Studies looking at different plant species have found some common bacterial groups (e.g. bacteria from the genus *Sphingomonas* and *Pseudomonas* tend to be abundant in flower samples; Wei & Ashman, 2018; Rebolleda-Gómez & Ashman, 2019) and some species of nectar yeast are common across systems and even different continents (e.g. *Metschnikowia reukaufii*; Pozo *et al.*, 2012; Herrera *et al.*, 2014; Dhami *et al.*, 2018). However, there is also significant variation in community composition across plant systems. For example, Junker & Keller (2015) found that Enterobacteraceae dominated the stamen and style samples of *Metrosideros polymorpha*, whereas Rebolleda-Gómez & Ashman (2019) found that these organs are dominated by other Proteobacteria (mostly α-proteobacteria) in *Mimulus guttatus*. Finer scale patterns are also emerging, such as differences between sexes within populations (Tsuji & Fukami, 2018; Wei & Ashman, 2018), across floral organs within a flower (Allard *et al.*, 2018; Rebolleda-Gómez & Ashman, 2019), and throughout the flower lifespan (Shade *et al.*, 2013), suggesting strong floral organ and trait-based filtering of microbial communities.

Despite uncovering these ecological patterns, we do not fully understand how floral microbes affect plant fitness, and the limited knowledge available stems exclusively from nectar microbes and floral pathogens. Furthermore, if floral traits exert selection on microbial communities, then these could feedback to affect the evolution of floral traits, initiating an eco-evo feedback within the anthosphere. In this viewpoint, we explore the potential effects of microbes on floral evolution by integrating current knowledge of the anthosphere and evidence from other plant–microbe interactions into an established evolutionary framework on multilevel

selection (Wolf *et al.*, 1999; Bijma & Wade, 2008; Hendry, 2016). In doing so, we reveal exciting avenues for understanding floral evolution and elucidate experimental approaches that will accelerate answering key questions regarding the anthosphere. Ultimately, we seek to understand how the evolution of floral traits could feedback and shape ecological interactions between flower and microbes.

Floral extended phenotype

Spatial and temporal patterns of variation in floral microbes suggest that floral traits can affect the microenvironmental conditions and resources available for microbes, and thus their establishment and potential to evolve. Numerous floral traits might influence microbial persistence and growth in the anthosphere, including floral morphology (Herrera, 2005; Fig. 1a–d), petal pigments (Gronquist *et al.*, 2001; Koski & Ashman, 2015; Fig. 1e–g), petal cell shape (Whitney *et al.*, 2011; Esser *et al.*, 2015; Fig. 1h), volatile production (Boachon *et al.*, 2019), and reward or secondary metabolite chemistry (Thornburg *et al.*, 2003; Huang *et al.*, 2012), and thus act as environmental 'filters' on floral microbial communities. Resource quality of organs within flowers can also change over time thereby affecting the probability of establishment, growth and survival of different microbes. Resource quality traits, such as nectar and pollen quantity, petal color and volatile production, also change as flowers age (e.g. Ashman & Schoen, 1994; Weiss, 1995; Raguso & Weiss, 2015). Similarly, sexual and mating systems influence plant allocation to many of these floral traits (Ashman *et al.*, 2005; Goodwillie *et al.*, 2010) leading to pronounced polymorphisms within taxa that can influence microbial populations and communities. For instance, diversity and composition of microbes differs between male and female flowers (Wei & Ashman, 2018) and nectar (Tsuji & Fukami, 2018), which may be driven in part by sex-differential allocation of resources to petals, pollen and nectar.

The effects of floral traits on microbial survival and growth, however, have rarely been tested outside of nectar composition (e.g. Herrera *et al.*, 2010; Pozo *et al.*, 2012) or in taxa other than pathogenic microbes (see Farkas *et al.*, 2012; McArt *et al.*, 2014 and references cited therein). Nevertheless, these studies show that floral nectar traits can act as highly selective environments that favor the establishment of a limited number of microbial taxa (Herrera *et al.*, 2010; Pozo *et al.*, 2012; Dhami *et al.*, 2018). Likewise, temporal variation in floral trait expression can drive microbe population dynamics, for instance the exudates of stigmatic papillae of apple and pear flowers diminish as the flowers age, and eventually cease to sustain the growth of the pathogen *Erwinia amylovora* (Thompson & Gouk, 2003).

The presence of microbes in flowers can in turn contribute to floral phenotype via microbe-expressed traits or microbial modification of plant trait expression. Nectar chemistry (e.g. amino acids, sugars, pH) is changed by the presence of microbes (reviewed in Parachnowitsch *et al.*, 2018), and can differ depending on the microbe (e.g. Rering *et al.*, 2018; Russell & Ashman, 2019). Microbes also produce volatile compounds that contribute to total floral bouquet, and metabolically modify plant volatiles (Peñuelas

et al., 2014; Helletsgruber *et al.*, 2017; Rering *et al.*, 2018). Along with chemical contributions to floral phenotype, microbes can modify the visual characteristics of flowers directly (e.g. potentially as a result of pigment production or fluorescence from bacterial pyoverdine; Hendry *et al.*, 2018) or indirectly (e.g. by inducing changes in petal colors or their longevity; Engelhard, 1970; Jennersten, 1988).

Finally, similar to numerous studies of leaves and roots (e.g. Horton *et al.*, 2014; Edwards *et al.*, 2015), evidence is beginning to accumulate that plant pheno(geno)type can affect floral microbial communities (e.g. Allard *et al.*, 2018; Steven *et al.*, 2018; Wei & Ashman, 2018), leading to the potential for covariance between floral trait values and microbial taxa or functional traits. Viewed in this way, one can see that nonrandom trait–microbe associations have the potential to create an extended floral phenotype that is subject to evolution.

Evolution of the extended floral phenotype: multilevel selection models

Floral traits can be associated with functional or taxonomic aspects of microbial communities, thus raising the possibility that the extended floral phenotype (i.e. the resulting phenotype from microbe–plant interactions; Fig. 2) evolves. For evolution of this extended phenotype to occur, floral traits must covary with microbial community composition (or function), this association must be heritable and lead to differences in plant fitness. Multilevel selection theory provides a framework for quantifying the joint effect of plant and microbe on evolution by formalizing (1) the contributions of floral microbes to floral phenotypes, (2) heritable variation in flower–microbe associations, and (3) selection parameters that influence the rate and trajectory of floral trait evolution (e.g. Goodnight *et al.*, 1992; Wolf *et al.*, 1999; Bijma & Wade, 2008). Contextual analysis designed to distinguish group-level (or community-level) selection from individual-level selection (Goodnight *et al.*, 1992) has been applied to interacting groups within taxa and between taxa (Goodnight, 2011). In this context, we can recognize individual-level selection acting only on the plant (without its associated microbes) as separate from selection at the group-level, that is, acting on the plant and its associated microbial community – the extended phenotype.

Using contextual analysis, we can recognize the role microbes play in floral evolution by decomposing the observed floral phenotype into the direct effects of plant genotype and associative effects of its floral microbial community (adapted from Bijma & Wade, 2008). For example, the floral volatile profile of plant i (z_i) is the result of the volatile compounds produced by that plant's genotype ($Z_{D,i}$; direct effects) and the volatile compounds produced by plant i in the presence of a particular floral microbial community j of plant i (Z_{ji}; associative effects; Fig. 2a). For simplicity in this viewpoint, we consider the effects of the whole community, but acknowledge that the phenotypic contributions of this microbial community (if they are additive) can be decomposed into the effects of the sum of all of the N constituent microbes ($Z_{ji} = \sum_{n=1}^{N} Z_{n,i}$). In addition, if plant and microbe interact, then the phenotype could also include components that can only result
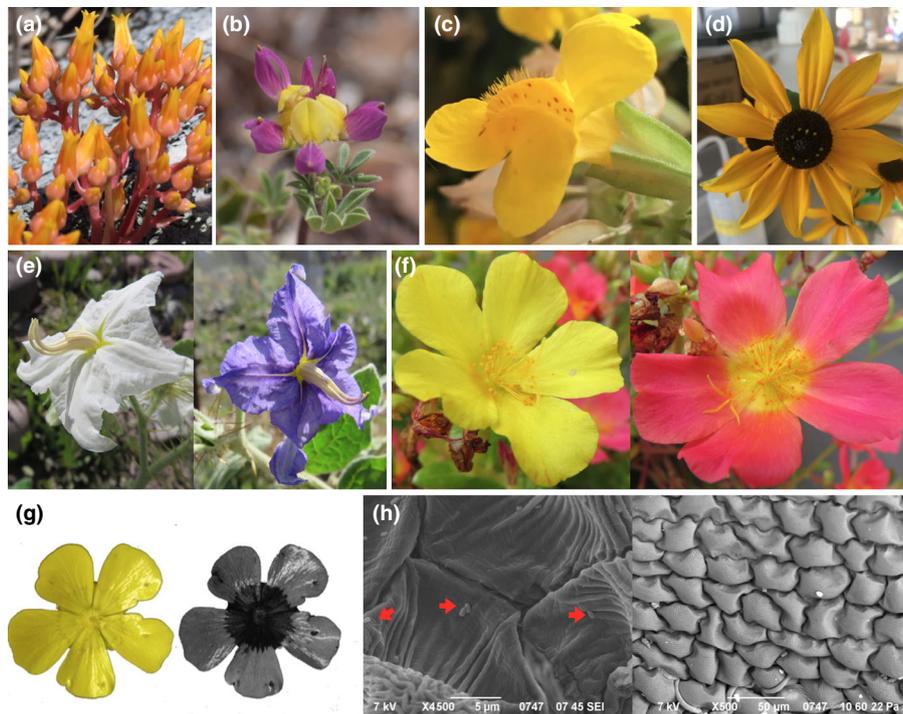
Fig. 1 Exemplar floral traits that could affect microbial community composition. (a–d) Different floral morphologies, as demonstrated by (a) *Dudleya cymosa*, (b) Harlequin lupin (*Lupinus stiversii*), (c) a close up of petal trichomes of the yellow monkeyflower (*Mimulus guttatus*), and (d) a composite inflorescence of (*Helianthus tuberosus*). (e–g) Pairs of images highlighting floral coloration patterns. Color polymorphism in (e) *Solanum houstonii*, (f) *Portulaca oleracea*, and (g) human visual and UV image of the meadow buttercup *Ranunculus acris*. (h) Scanning electron microscopy (SEM) images of petal epidermis cells (right – *Helianthus tuberosus*, left – *Verbesina alternifolia*). In (h) it is possible to see epiphytic microbes (red arrows).
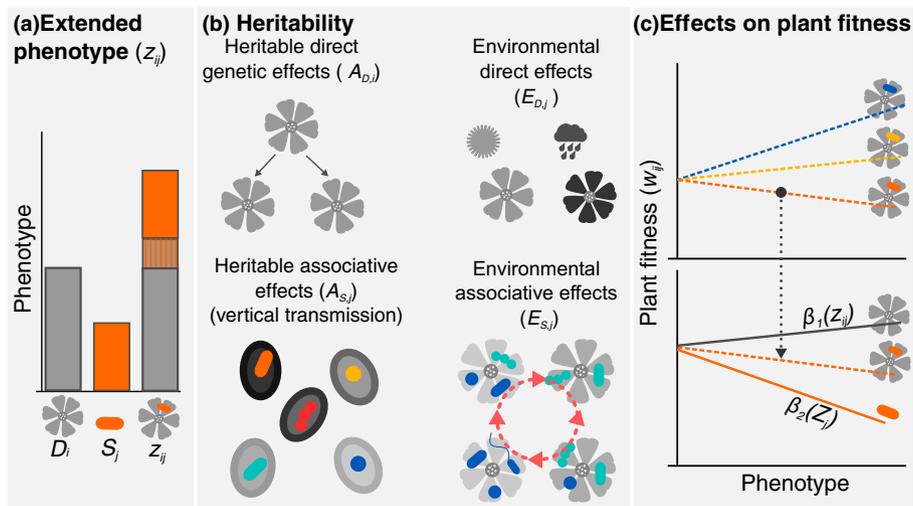

Fig. 2 Multilevel selection framework for understanding evolution of the anthosphere. (a) Floral phenotype of plant *i* (Z_{ij}) is the result of traits produced by plant genotype *i* (D_i; direct effects) and those produced by plant *i* with a particular microbial community *j* (S_j; associative effects) including interactions between microbe and plant (hatched bar). (b) The phenotype of the plant *i* (Z_{ij}) can further be decomposed to account for heritable direct genetic effects ($A_{D,i}$) and environmental direct effects ($E_{D,i}$) produced by plant genotype *i* (top), as well as the heritable associative ($A_{S,i}$) and environmental associative effects ($E_{S,i}$) produced by plant *i* in response to its floral microbial community *j*. Pollinator preferences and the filtering effect of floral traits can reinforce nongenetic associations. See microbial heritability section for more detail. (c) The total selection gradient on a floral phenotype can be attributed to the selection (β_1) via the plant trait and selection (β_j) via plant *i*'s microbial community *j*. Assuming additivity, community effects can be decomposed as the contributions of individual microbes. See microbes can affect floral fitness section.

from the interaction and that are not produced by either of the partners alone (hatched bar, Fig 2a). For instance, when microbes take up the plant's compounds and release them in a modified configuration.

Microbial heritability

Microbial community heritability (or broad community heritability) can be defined as the association between microbial community

components (e.g. the presence and absence of certain species) and the plant genotype (*sensu* Johnson & Stinchcombe, 2007). Thus, the plant extended phenotype can then be decomposed into heritable and nonheritable components (Bijma & Wade, 2008). For example, the observed floral volatile composition of plant i (z_i) is the result of heritable direct genetic effects ($A_{D,i}$) and environmental direct effects ($E_{D,i}$) on the volatile compounds produced by plant genotype i, as well as the heritable associative ($A_{S,ji}$) and environmental associative effects ($E_{S,i}$) on the volatile compounds produced by plant i in response to its floral microbial community j (Fig. 2b).

Heritability of microbial communities (i.e. the association of plant genotypic variation and variation in microbial communities across a generation) can come about through different mechanisms. Some microbes will be transmitted vertically, some horizontally, and some via a combination of both methods. Heritable associative effects ($A_{S,ji}$) can occur through vertical transmission of floral microbes. Endophytic microbes can be vertically transmitted via seeds or pollen (Compant *et al.*, 2011; Nelson, 2017). Some microbes inoculated in flowers, for example, can be transferred to the next generation in the seed (Mitter *et al.*, 2017). Similarly, epiphytic microbes that attach to persistent floral tissues during fruit development (e.g. styles or sepals) can be transported during seed dispersal (reviewed in Nelson, 2017). However, this form of transmission is less consistent over time (e.g. Afkhami & Rudgers, 2008; Barret *et al.*, 2016). Reliability of transmission will affect the impact of microbes on floral evolution.

Overall, selection on floral traits through microbial effects on plant fitness will be more efficient if the whole microbial community and its interactors are transmitted together (i.e. high levels of 'community heritability'; Goodnight, 2011). Of course, whole microbiomes are unlikely to be transferred entirely together. Nevertheless, evolution of floral traits can still occur as long as floral traits can reliably select for and/or against those microbial traits that have strong fitness consequences, preserving an association between plant genetic variation and the extended phenotype resulting from these microbial interactions.

In the anthosphere, dispersal and interaction of microbes across flowers can enhance associations between flowers and microbial communities. These associations can increase the broad community heritability (through co-dispersal of different species) thereby increasing the efficiency of group selection on microbe and floral evolution (Goodnight, 2011). Vertical transmission of microbes is further augmented by the generation of 'community-level' heritability that results from spatial associations between plant genotypes and microbial strains. Finally, multi-generational associations between flowers and microbes can result from flowers functioning as environmental filters that select for certain microbial species, or, at least, microbial functions with strong fitness consequences (Fig 2b).

Environmental associative effects ($E_{S,ji}$) could be consistent across plant generations, leading to repeatable assembly of floral microbial communities (Fig. 2b). And while these associative effects do not evolve, they can shape the adaptive landscape of the plant. Associations between microbes and their hosts can be maintained and regulated through sanctions, resource allocation,

and other mechanisms of host control (e.g. Schluter & Foster, 2012). Plants could increase the repeatability of specific microbes associating with flowers via selective allocation and sanctioning mechanisms that regulate nonbeneficial vs beneficial strains, as in roots and leaves (e.g. Afkhami & Rudgers, 2008; Sachs *et al.*, 2010), or by favoring strains with strong priority effects (i.e. arriving early and altering the environment for later arriving microbes; Toju *et al.*, 2018). For example, apple flowers select for bacteria that antagonistically interact with other strains, thereby excluding the latter (Steven *et al.*, 2018). These priority effects can persist over time, ultimately shaping the entire microbial community (Toju *et al.*, 2018). And while we often think of priority effects as increasing contingency, strong filtering that favors initial colonization by a few key microbial species may have downstream effects that structure floral microbial communities, and potentially increase community-level associations. As long as floral traits are heritable, and the association between these traits and the microbial community is stable over time (with similar microbial communities, or at least similar functions and fitness effects maintained through generations), then we can expect the evolution of floral traits in response to their associated microbiomes.

Microbes can affect floral fitness

Floral microbes have the potential to mediate the relationship between floral traits and plant fitness directly by affecting reproduction and indirectly through altering floral interactions with other organisms (Huang *et al.*, 2012; Vannette *et al.*, 2013; Egamberdieva *et al.*, 2017). We can quantify these effects by partitioning plant fitness (w_{ij}) (i.e. the fitness of plant i associated with microbial community j) into selection gradients estimated by individual (β_1) and associative (β_2) components (Fig. 2c; Bijma & Wade, 2008).

Microbes might directly impact plant fitness through altering plant reproduction. As discussed previously, microbes can produce various phytohormones (e.g. auxin, cytokinins, gibberellin, antheridiogens) that are known to affect plant growth and development (Egamberdieva *et al.*, 2017; Olanrewaju *et al.*, 2017; Park *et al.*, 2017; Ganger *et al.*, 2019). One of the best examples of microbial direct effects on plant fitness is the complete sterilization of anthers of *Silene* flowers by the anther smut fungus (*Microbotryum*; Biere & Antonovics, 1996). Floral microbes can also directly affect fitness by inhibiting germination of pollen (Eisdcowitch *et al.*, 1990). While little is known about the direct effects of nonpathogenic microbes on plant fitness, microbes from soil have been shown to affect fitness through inducing changes in flowering time (Lau & Lennon, 2011; Wagner *et al.*, 2014).

In addition, microbes can affect plant fitness indirectly by modifying how flowers interact with other microbes or pollinators. Microbes that occupy floral structures may impose physical barriers to the establishment and proliferation of other microbial taxa, such as pathogens (Olanrewaju *et al.*, 2017). Furthermore, floral microbes can alter the quality of floral cues and rewards (e.g. nectar, floral volatiles), which are key components of pollinator attraction and foraging behaviors. For example, nectar contaminated by yeast (and sometimes bacteria, see Junker *et al.*, 2014) is

frequently attractive to pollinators such as bees (e.g. Good *et al.*, 2014; Herrera *et al.*, 2013), resulting in preferential visitation (Schaeffer & Irwin, 2014) and even enhancement of components of male fertility (Schaeffer & Irwin, 2014). Likewise, bees perceive (Rering *et al.*, 2018) and can learn floral microbial scents against a floral background (Russell & Ashman, 2019), suggesting that much like flower cues (Schiestl & Johnson, 2013), the presence of microbial cues on flowers may enhance pollinator visitation and facilitate pollen transfer.

Microbial effects on floral traits may be synergistic or antagonistic to the flower's own cues, thereby altering selection gradient parameters and shaping the relationship between floral traits and plant fitness (Fig. 2c). Specifically, floral microbial communities may strengthen or weaken a positive relationship between a floral trait and plant fitness or change the direction of the selection gradient entirely (Fig. 2c). For example, floral microbial cues might mask floral cues that pollinators use to ascertain floral reward presence, causing pollinators to make more visits to ensure they acquire a floral reward. These indirect effects of floral microbes on pollinator behavior would strengthen the relationship between floral cues and plant fitness by enhancing pollen dispersal at a cost to the pollinator. Although selection gradients on the extended floral phenotype have yet to be estimated in any system, multilevel selection models allow the role of floral microbial communities in selection to be evaluated and contrasted with plant contributions, similar to previous work highlighting the potential for soil microbes to alter selection gradients in plants (e.g. Lau & Lennon, 2011; Wagner *et al.*, 2014).

Types of floral phenotypes that could be subject to these dynamics and why

Here, we illustrate the potential effects of microbes on floral trait evolution, using scent and color as exemplars. Then, we consider how studies of key plant reproductive strategies (mating and sexual system) may also benefit from the perspective developed earlier.

Floral scent

The complex mixtures of volatiles produced by flowers can affect microbial community composition (Boachon *et al.*, 2019). Floral microbes can add scent compounds to flowers, and alter floral scent emission by inducing, reducing and/or even catabolizing floral chemistry (Peñuelas *et al.*, 2014; Helletsgruber *et al.*, 2017; Burdon *et al.*, 2018, Cellini *et al.*, 2019). Microbially-mediated changes to key floral volatiles (e.g. linalool) potentially alter pollinator behavior (Burdon *et al.*, 2018; Cellini *et al.*, 2019), and indeed pollinators such as bees can perceive (Rering *et al.*, 2018), respond innately, and learn to prefer or avoid microbial scents on flowers (Russell & Ashman, 2019). Pollinator preferences mediated by floral cues are key drivers of plant fitness and floral evolution (Schiestl & Johnson, 2013), thus epiphytic microbes that alter or enhance floral scent may shape plant fitness (Helletsgruber *et al.*, 2017; Burdon *et al.*, 2018; Russell & Ashman, 2019). These effects could lead to strong associations between microbes and plant phenotypes, which would enhance the effectiveness/fidelity of

pollinators. Flower scent, thus, seems a strong target for multilevel selection.

Floral color

Similar to scent, flower coloration affects important aspects of the microbial environment. Flower colors are produced by a combination of pigments and structural coloration, primarily due to cell shape (Noda *et al.*, 1994). Floral pigments can change the chemical composition of the flower, often affecting volatile emissions (e.g. Majetic *et al.*, 2007) or the resource base for microbes. Whereas structural coloration can affect the spatial structure of the flower (Fig. 1h) by shaping the diffusion of nutrients, light scatter, and wettability (Whitney *et al.*, 2011). Together, chemical and structural colors affect the temperature inside the flower (Whitney *et al.*, 2011), as well as the intensity of UV radiation (Koski & Ashman, 2015). Microbes that can withstand these micro-environmental conditions could, in turn, affect coloration of the extended floral phenotype by their presence or via their effects on floral color development (Engelhard, 1970; Cárdenas Flores *et al.*, 2007). Flower color has been associated with vulnerability to pathogens (Frey, 2004), and can mediate fitness through changes in pollinator visitation (Weiss, 1995). As is the case with pollinator scent preferences, pollinator color preferences are likely to affect not only plant fitness, but also the degree of association between particular floral genotypes and microbial community composition, suggesting that floral color could also be subject to multilevel selection.

Mating/sexual systems

Mating and sexual systems of flowering plants could also be subject to multilevel selection because they can impact flower–microbe interactions, expression of extended phenotypes and selection. Variation in floral allocation between sexual morphs, or among selfing/outcrossing morphs, will create different habitats and potentially varying filtering strength for microbes within populations. For instance, females of dioecious species often produce less nectar and/or floral volatiles than males (Ashman, 2009), and thus provide harsher, potentially more selective, floral environments than males. Likewise, selfing morphs provide fewer and more ephemeral resources for microbes than outcrossing ones (Goodwillie *et al.*, 2010). Flower and leaf microbiomes of male and female plants of dioecious species can differ strongly, suggesting a role for sexually-dimorphic filtering (e.g. Wei & Ashman, 2018; Wu *et al.*, 2019), although much less is known about plants that vary in mating system.

Morph-specific communities of microbes, or differential responses to the same microbes, could produce divergent extended phenotypes within populations. For instance, sex-specific responses to pathogenic microbes have been observed, and these responses can lead to changes in the sexual phenotype of the plant (e.g. anther smut fungus induces partial sex change in *Silene latifolia*; Zemp *et al.*, 2015). Although not yet studied in angiosperms or in flowers, a recent study found that soil bacteria can mediate sex changes in fern gametophytes (Ganger *et al.*, 2019), suggesting that microbes possibly play a role in plant sex expression more broadly.

Heritability of microbes may also vary with mating and sexual system. For instance, in dioecious species only females produce seeds, so heritability of microbes will only be relevant between female flowers and fruits. There is covariation between the core microbiome of flower and fruit/seeds in hermaphroditic species, such as tomato and oil seed rape (Allard *et al.*, 2018; Prado *et al.*, 2019). While no data is currently available for wild dioecious species, overlap between flower and fruit microbiomes was similar for male sterile and hermaphrodite oil seed rape plants (Prado *et al.*, 2019), suggesting a preeminence of the ovary microbiome to the association. We might also predict higher heritability for floral microbes in selfing species than outcrossing ones because reduced interactions with pollinators could lead to a less variable and possibly less complex community. Indeed, a recent study on oil seed rape showed that seeds from autonomously pollinated flowers had less variable microbial communities than insect pollinated flowers (Prado *et al.*, 2019). Likewise, in tomato, excluding pollinators reduced variation in microbial communities among flowers and fruits (Allard *et al.*, 2018). More data is needed, particularly in systems with natural variation in mating system, such as those with cleistogamous and chasmogamous flowers, to fully test the idea that selfers have higher heritability of floral microbiomes than outcrossers.

Finally, microbial contributions to floral phenotype may be under stronger selection via male rather than female fertility as siring success is often limited by access to mates, whereas female fertility is limited by resources provided pollen is not limiting (e.g. Ashman & Morgan, 2004). Thus, sex-specific formulations of selection decomposition (Fig. 2a–c), and those that consider the shape of fitness gain curves (e.g. Charnov, 1979; Ashman & Morgan, 2004) will be necessary to fully understand impacts of microbes on sexual system evolution.

Future directions and approaches

As knowledge of ecological interactions between flowers and their microbial communities expands, it becomes increasingly important to consider the evolutionary impacts of these associations as well as their potential eco-evo dynamics. Through synthesizing current knowledge of the anthosphere within a multilevel selection framework, we illustrate the potential for the extended floral phenotype to evolve and describe various pathways by which this may occur. Yet, many gaps in knowledge remain and warrant explicit investigation. Here we enumerate goals for future directions and valuable approaches to take.

Floral extended phenotype

• Extend our understanding of floral microbes beyond nectar, to epiphytic microbial communities of other floral tissues.
• Evaluate effects of floral variation (e.g. volatile composition, color) on microbial community composition, microbe population sizes, and growth rates.
• Functionally characterize microbial communities and determine whether these functions (e.g. volatile production) vary with floral phenotype, sexual polymorphism, or plant taxonomic affinity.

• Partition floral phenotype (e.g. volatile profile) into microbial and plant components.

Selection on the extended floral phenotype

• Determine the general and specific effects of microbes on plant fitness via their effects on pollinator visitation and subsequent effects on pollen receipt and donation.
• Determine general and specific effects of microbes on post-pollination processes, including pollen tube growth, seed fertilization, and maturation.

Microbial heritability

• Determine the rate of vertical transmission of epiphytic microbes, as well as compositional overlap (covariance) between flower, fruit, and seed microbial communities, and their consistency over time.
• Determine whether selective visitation by pollinators reinforces floral microbial communities locally (or over time), thereby creating environmental associative effects.
• Evaluate the contributions of pollinator preferences, learning, and site fidelity to pollinator-mediated environmental associative effects.

Controlled experiments that manipulate flower phenotypes, microbial communities, and pollinator visitation hold immense promise for answering fundamental questions concerning the creation of extended floral phenotypes and their fitness consequences. For instance, applying synthetic communities of known microbes to phenotypically variable flowers in the glasshouse will be useful for evaluating how specific floral traits filter or shift microbial communities. When paired with control plants, this approach can also address fitness impacts of floral microbiota generally. By contrast, application of single microbes will allow pinpointing of specific fitness effects of microbial taxa on pollinator visitation (e.g. Russell & Ashman, 2019) or pollen dispersal and seed production (e.g. Schaeffer & Irwin, 2014). One can also leverage pollinator behavior to elucidate patterns of microbe transfer (e.g. Russell *et al.*, 2019) and environmental associative effects by adding a common pool of microbes to flowers and evaluating the correlation between microbial community composition of flowers and fruits for plant with and without pollinator visitation. Finally, selective passaging experiments, such as those used in whole tomato plants (e.g. Morella *et al.*, 2019), will be powerful for elucidating microbial evolution in response to floral phenotype.

In addition, a variety of mechanistic approaches will be useful for future research on the anthosphere. In particular, functional assays, metabolic and molecular analyses (e.g. Boachon *et al.*, 2019), as well as classical microbiology techniques (e.g. evaluating effects of UV radiation on phyllosphere bacteria, Jacobs & Sundin, 2001) will aid in understanding microbial responses to flower environments and microbial contributions to the extended phenotype. Furthermore, quantitative genetic approaches to characterizing plant variation (genome-wide association study (GWAS), quantitative trait locus (QTL)), such as those applied to the phyllosphere and rhizosphere, will be useful for associating loci relevant to flower

and fruit development with microbial taxa. For instance, the GWAS of *Arabidopsis thaliana* accessions identified important loci (relevant to trichomes and defensive hormones) associated with dominant bacterial taxa in the phyllosphere, and uncovered high heritability (46%) of these (Horton *et al.*, 2014). Lastly, biochemical and reverse genetics of floral phenotype can reveal specific plant traits that influence microbial populations. For instance, Boachon *et al.* (2019) determined petal sesquiterpene production controlled stigmatic bacterial populations. This approach could be used more broadly to identify traits responsible for epiphytic filtering across floral organs and floral and fruit traits that facilitate vertical transmission. A full understanding of microbial effects on floral evolution will ultimately be gained by applying a combination of phenomenological and mechanistic approaches.

Conclusion: eco-evolutionary feedbacks in the anthosphere

In this viewpoint, we argued that microbes play an important role in floral evolution and highlighted open questions and new avenues of research. Rapid evolutionary change in floral traits and plant reproduction can, in turn, affect the ecology and evolution of microbes in the anthosphere, creating a feedback between evolutionary change and ecological dynamics (Hendry, 2016). Although we focused our attention on the evolution of plants in response to their floral microbes, microbe–microbe interactions can shape the ecology and evolution of microbes (Koskella *et al.*, 2011) and their interactions with flowers and pollinators. Finally, the anthosphere is a quintessential microcosm of eco-evolutionary dynamics because of the interplay of all these players (microbes, plants, pollinators). Including these players may reveal complex multi-player eco-evolutionary dynamics. For example, it is reasonable to speculate that microbes will evolve to manipulate pollinators for their dispersal, and if the movement of these microbes is correlated with pollen deposition, then plant genotypes favoring the growth of these microbes could increase in frequency. Understanding the impact of microbes on plant reproductive ecology represents a new and exciting frontier for understanding angiosperm evolution.

Author contributions

MR-G and T-LA led discussions and designed the overall framework. MR-G, T-LA and NJF wrote the manuscript with input from ALR and NW. All authors contributed to discussions.

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