

Review



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Potential effects of nectar microbes on pollinator health

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Floral nectar is prone to colonization by nectar-adapted yeasts and bacteria via air-, rain-, and animal-mediated dispersal. Upon colonization, microbes can modify nectar chemical constituents that are plant-provisioned or impart their own through secretion of metabolic by-products or antibiotics into the nectar environment. Such modifications can have consequences for pollinator perception of nectar quality, as microbial metabolism can leave a distinct imprint on olfactory and gustatory cues that inform foraging decisions. Furthermore, direct interactions between pollinators and nectar microbes, as well as consumption of modified nectar, have the potential to affect pollinator health both positively and negatively. Here, we discuss and integrate recent findings from research on plant-microbe-pollinator interactions and their consequences for pollinator health. We then explore future avenues of research that could shed light on the myriad ways in which nectar microbes can affect pollinator health, including the taxonomic diversity of vertebrate and invertebrate pollinators that rely on this reward.

This article is part of the theme issue 'Natural processes influencing pollinator health: from chemistry to landscapes'.

1. Introduction

A diversity of pollinators ranging from tiny thrips to large lemurs seek nectar to fuel their energetic demands [1–3]. This energy is derived primarily from metabolism of chemical constituents, namely the sugars glucose, fructose, and sucrose [4–6], together comprising 6–85% (w/v) of nectar [7], along with lower concentrations of amino acids, lipids, minerals, and vitamins [8–11]. Such resources are critical for reproduction of many animals, but animals are not the only organisms that benefit from floral nectar. Increasingly, nectar is being appreciated for its role as a habitat for archaea, bacteria, fungi, protozoa, and viruses that disperse to flowers via air-, rain-, and animal-mediated transmission [12–14].

Current research regarding nectar microbes largely centres on documentation of patterns of occurrence and diversity (reviewed in [14]), as well as consequences of microbial metabolism for plant fitness (reviewed in [15]). In this paper, we review the emerging evidence suggesting that the nectar microbiome can also influence pollinator health, defined here as a state of well-being that allows pollinator individuals and populations to achieve high fitness through increased longevity and reproduction even in the presence of pathogens [16]. The nectar microbiome could affect pollinator health in multiple ways, including modification of the nutritional landscape encountered by pollinators, altering nectar foraging behaviours, and interacting with symbionts and pathogens associated with pollinators. Many of the potential consequences discussed here are untested hypotheses. Thus, we first review current findings and then highlight future research areas that would improve our understanding of the ways in which nectar microbes affect pollinator health.

2. Occurrence, abundance, and diversity of nectar microbes

To date, surveys of the nectar microbiome have largely focused on bacteria and fungi [17–19]. Generally, both microbial occurrence and abundance in nectar

increase over the lifetime of a flower [14,20], with yeasts and bacteria reaching densities of up to about 10^5 and 10^7 cells μl^{-1} , respectively [18,19]. Nectar microbial communities tend to be species-poor, at least in temperate regions, with only one yeast or bacterial species achieving dominance [21,22]. Dispersal limitation [21], competitive exclusion [20,23], and the environmental constraints of nectar [24], including high osmotic pressure and low nitrogen availability, appear to contribute to low species richness. Subtropical and tropical plant communities, however, may support a higher species richness of nectar microbes [25,26], perhaps owing to longer periods of habitat availability [27], greater plant species richness [27,28], and the short longevity of tropical flowers [29], preventing competitive exclusion among species of nectar microbes.

Not surprisingly, taxa gaining dominance tend to be nectar specialists (i.e. animal-transported, osmotolerant species that are found almost exclusively on pollinators and in nectar) or autochthonous species *sensu* [30]. Nectar specialists include the ubiquitous yeasts *Metschnikowia reukaufii* and *Metschnikowia gruessii* and bacteria of the genera *Acinetobacter* and *Rosenbergiella* (reviewed in [14]). In addition, many non-specialists are frequently encountered in nectar, including fungi and bacteria that are associated with animals, other plant organs, or environmental habitats such as water or soil [31]. However, generalist species are not well-adapted to the stressful nectar environment and typically occur less frequently and appear at low abundances, while specialist microbes are common and found at much higher densities [14]. With high cell numbers, nectar specialists can metabolize many of the available nutrients in nectar and alter its quality. Finally, while found in lower abundances, non-specialist microbes encountered by nectar foragers, such as pathogens and gut symbionts, still have the potential to directly affect pollinator health.

Improved methodologies could shed light on the living microbes found in nectar that could have the ability to colonize pollinator hosts, food stores, and nesting habitats. The possible roles of microbial communities depend on how many microorganisms are present. One future priority should be to evaluate absolute community sizes, which can be assessed through various methods such as quantitative polymerase chain reaction (qPCR). Microbes must also be viable to colonize new hosts. For example, honeybee and bumblebee pathogens and gut symbionts are known to be transmitted via contact with floral surfaces, but the longevity of these microbes outside of their host organisms can be limited to a few hours or less [32]. To date, most nectar microbiomes have been described using PCR amplicons of 16S rRNA or internal transcribed spacer genes for bacteria and yeast, respectively. Therefore, the taxonomic profiles probably include entities that do not represent living cells. To ensure identification of only viable microbes, one method currently used in nectar microbiome research is to first culture dilutions of nectar samples on agar plates and then sequence individual colonies with unique morphotypes. This method is useful for identifying nectar specialists, which are largely culturable, but is probably less effective for identifying viable cells of non-specialist microbes. Another method to detect living cells is to start with RNA samples since RNA degrades quickly when a cell is lysed [33]. This approach has seen little use in nectar microbiome research and might help to elucidate the findings of studies on nectar communities and their effects on pollinators.

Nectar microbes are encountered by pollinators in flowers of nearly all angiosperms surveyed thus far. Their effects on the nutritional quality and attractiveness of nectar have been increasingly studied over the last two decades. In the next section, we outline the known effects of several common nectar microbe species on nectar chemistry.

3. Nectar microbes' influence on nectar chemistry

Nectar microbes, particularly specialists, affect the nutritional quality of nectar through consumption of mono- and disaccharide sugars, alterations of their relative proportions, and decreases in pH through fermentation [34–37]. Additionally, they can metabolize, modify, or synthesize amino acids and secondary metabolites, resulting in altered nectar flavours and scents that can be perceived by pollinators and impact pollinator preferences [38–40]. The chemical signatures of several species of microbes isolated from nectar have largely been assessed *in vitro*, discussed more below.

Nectar-specialist yeasts in the *Metschnikowia* genus tend to reduce the concentrations of sucrose and glucose, leaving an elevated proportion of fructose in nectar [17,36,41,42]. These changes are associated with production of volatile organic compounds (VOCs) detectable by floral visitors, including alcohols, esters, and ketones [40,43,44]. Moreover, these yeasts deplete amino acids and possess a relatively high number of genes related to nitrogen scavenging in nitrogen-poor environments like the nectar habitat [45]. *Metschnikowia reukaufii* has been found to reduce nectar pH by a half unit in 36 h and more than two units in a 5-day period [34,35]. The effects of a handful of non-specialist fungi on nectar chemistry and VOC emission have also been assessed. The generalist black fungus, *Aureobasidium pullulans*, has a minimal effect on amino acids, but can reduce sucrose by 90% [43]. This species also appears to metabolize fructose, with resulting nectar containing a high proportion of glucose [43]. In comparison, the phylloplane yeast, *Sporobolomyces roseus*, has little effect on sugar concentration but can increase amino acid concentrations by 12%, on average [43]. On the other hand, *Hanseniaspora uvarum*, an apiculate yeast common on mature fruits, does not significantly alter concentrations of sugars or amino acids but has been shown to significantly alter nectar VOC emissions by producing acetaldehyde and small alcohols [40,43]. The three non-specialist fungi studied can also reduce nectar pH by 0.8 (*Ha. uvarum*) to 1.8 (*Au. pullulans*) in a 7-day period [43].

Bacterial nectar specialists profiled thus far include *Neokomagataea* sp., *Rosenbergiella nectarea* and flower-associated species of the *Acinetobacter* genus. *Neokomagataea* sp. and *R. nectarea* have metabolic effects similar to *M. reukaufii*, reducing sucrose and increasing glucose and fructose concentrations [37,40,46,47]. Flower-associated *Acinetobacter* species consume fructose and nitrogen-containing byproducts of yeast metabolism such as ammonia and have been found to associate with yeasts in nectar [48]. *Rosenbergiella nectarea* and *Acinetobacter* species reduce amino acid concentrations, while *Neokomagataea* increases amino acids. Non-specialists in metabolic experiments include *Lactococcus garvieae*, *Apilactobacillus kunkeei* [49], *Erwinia tasmaniensis*, and *Asaia* species among others [37,40,50,51]. *Erwinia tasmaniensis* does not change nectar sugars or amino acids but

reduces nectar pH by one unit over 4 days. The acetic acid bacteria *Asaia platycodi* and *Asaia astilbes* reduce sucrose and amino acid concentrations, increase monosaccharide concentrations, and significantly reduce nectar pH via production of acetic acid and other metabolic byproducts. The lactic acid bacterium *L. garvieae* has no effect on sugars, increases amino acid concentrations, and reduces nectar pH with production of lactic acid and other metabolites [37]. In general, bacteria share many of the same VOCs produced by fungi, albeit emitted in lower amounts [40]. Unique compounds have been detected, however, including 2,5-dimethylfuran, emitted by *As. astilbes* and *Neokomagataea* sp. [40].

From these studies, it is clear that nectar microbes can significantly modify nectar chemistry along several different axes that could impact pollinator foraging. Having established these microbial effects, we now discuss current research on how microbe-induced changes to nectar aroma and nutritional content alter pollinator foraging decisions and subsequent fitness (figure 1). Where appropriate, we also highlight future research areas that would improve our understanding of the ways in which nectar microbes affect pollinator health.

4. Current research and future directions on nectar microbe effects on pollinator health

(a) Microbially induced effects on pollinator foraging

Pollinators use visual, olfactory, and gustatory cues to inform foraging decisions. Thus, it may not be entirely surprising that microbe-induced shifts in nectar chemistry can affect pollinator preferences. With respect to scent, both bumblebees and honeybees are responsive to VOCs produced by nectar yeasts and bacteria. Indeed, naive *Bombus impatiens* and *Bombus terrestris* foragers can use VOCs produced by the yeast *M. reukaufii* to discover nectar [38,39]. Electroantennography studies have shown that bumblebee and honeybee olfactory neurons are sensitive to the microbe-produced VOCs *n*-hexanol, 2-ethyl-1-hexanol and 2-phenylethanol at low concentrations (0.4 μ mol) [40,52]. Additional VOCs identified that elicit a response in bumblebees and honeybees are 3-methylbutyl acetate and 2-nonane, respectively. Bumblebees also consume more yeast-inoculated nectar in laboratory and field experiments [38,39,52,53]. By contrast, bumblebees and honeybees have either an aversive or neutral foraging response to nectar bacteria, though this effect may depend on the identity of the bacterium [50,54] or sensory modality. For instance, *B. impatiens* has been observed to prefer odours produced by the bacterium *As. astilbes*, yet consumes more nectar colonized by *M. reukaufii* [39].

Despite these observed effects, the energetic consequences of microbe-induced altered nectar foraging for pollinator health remain largely unknown. Eusocial bees are sensitive to shifts in the identity and concentration of sugars in nectar. In honeybees, low nectar sugar (i.e. sucrose) concentrations are associated with a reduction in the number of foraging trips made by workers [55], probably informed via social communication (i.e. waggle dance) [56] and potentially translating to fewer resources for the colony. Bumblebees similarly have a strong preference for sucrose in nectar and will shift foraging tactics in response to shifts in concentration [57,58]. Yet, while nectar yeasts often

reduce sugar concentrations and particularly sucrose, bumblebees are attracted to nectar yeasts and incorporate more yeast-inoculated flowers into foraging bouts [38,53]. This evidence suggests that nectar microbes could supply benefits that outweigh the cost of diluted rewards.

Microbial scent cues could play a role in honest signalling of rewards since high abundances of nectar microbes are found in flowers with enough nectar to support them. Alternatively, nectar microbes could provide nutritional benefits (§4c). Yeasts might also warm nectar through metabolism and fermentation, particularly in winter-blooming plants like the herb *Helleborus foetidus* [59]. Given that some pollinators may prefer warmer nectar [60], perhaps because warmer nectar is easier to digest (but see [61]), nectar microbes may exert their influences on pollinator health through nectar warming. Viscosity is another aspect of nectar that can influence pollinator foraging, explaining 56% of nectar imbibition rate by bumblebees [62]. In the absence of microbes, sugar concentration and composition are significant predictors of viscosity [62]. Thus, nectar microbes could change viscosity indirectly via alterations to sugars. Microbial cells might also directly influence viscosity when microbial abundances are high. All of these mechanisms remain almost entirely hypothetical.

When there are multiple species of angiosperms in a landscape, the changes that nectar microbes make to pollinator foraging behaviour can affect the total nutritional value of floral resources that pollinators acquire from the plant community [63]. What makes it both interesting and difficult to understand regarding this landscape-scale effect of nectar microbes on pollinator foraging is the feedback between the microbes and pollinators. Microbes may affect pollinators by changing nectar chemistry, but microbes are affected by pollinators in the first place because microbial movement among flowers is largely facilitated by pollinators [64]. The dynamics of this feedback may be key to understanding the role of nectar microbes in affecting pollinator foraging, and ultimately their health.

(b) Microbially induced effects on pollinator health

The above evidence suggests that nectar microbes could mediate the effects of nectar quality and floral phenotype on the energetics of pollinator foraging. Here we discuss the effects of diet quality on pollinator health. Amino acid and carbohydrate availability in food resources is an important determinant of animal health and fitness [65]. Indeed, changes in sugar and amino acid quantities are known to affect the well-being of bees, with nutrient limitation reducing immune responses to infection [66–68]. Bumblebee workers consume 7.5% more sugars in response to immune stimulation, indicating that they require additional energy intake when under disease stress [69]. Dietary regimes can also change bacterial community composition in the digestive tract of honeybees and bumblebees. In a recent study, high sucrose diets resulted in more diverse honeybee midgut communities, while diets high in glucose or fructose led to more diverse hindgut communities [70]. Given that the gut microbiome of social bees can greatly affect their host's health through both metabolization of carbon-rich food sources and modulation of the immune system [71–73], greater consideration of links between microbiomes of nectar and the host gut is warranted.

The metabolic activities of several common nectar microbe species, described in §3, have been shown to alter the concentrations of sugars and amino acids available to pollinators. There are three additional potential mechanisms by which they could change the nutritional properties of nectar. First, microbes can modify the nutritional value of not just nectar *per se*, but also pollen that falls in. From observations of nectar droplets under the microscope, Herrera [74] described that the yeast *M. reukaufii* was often found clustered around pollen grains and hypothesized that the yeast might scavenge leached nutrients [74,75]. *Metschnikowia reukaufii* has also been found to induce pollen bursting, although this effect may depend on the plant species [76]. Further, Christensen *et al.* [77] showed that common nectar bacteria *Acinetobacter* spp. have the capacity to induce both germination and bursting of *Eschscholzia californica* (California poppy) pollen [77]. The nutrients released by pollen bursting may enhance the nutritional value of nectar for pollinators as well [78]. However, this possibility remains to be fully investigated. Second, some species of nectar yeasts and bacteria greatly reduce levels of hydrogen peroxide in nectar [34]. This effect could have a potential benefit to bee health as hydrogen peroxide significantly reduces bee survival and has effects on the bee gut microbiome [79]. Third, when consumed by pollinators, the microbes themselves, particularly yeasts, may serve as a modified form of nectar nutrition (e.g. vitamins and steroids [80]), as seen in non-pollinating *Drosophila* species [81–84]. These mechanisms remain almost entirely hypothetical.

When nectar microbes modify the nutritional value of nectar, this modification might affect not just the nectar-foraging adults themselves, but also egg production and the health of their offspring [85]. In honeybees and bumblebees reared in laboratory settings, diet quality has been shown to affect ovarian development and egg-laying in queens and queen-less workers [67,86,87]. Using queen-less bumblebee microcolonies, Schaeffer *et al.* [39] found that consumption of nectar inoculated with *M. reukaufii* does not affect egg production by female workers, despite their preference for *M. reukaufii*-inoculated nectar [39,52]. At the colony-level, however, Pozo *et al.* [36,88] revealed that the development of entire bumblebee (*B. terrestris*) colonies was affected by yeasts and bacteria in nectar, but these effects depended on the species added. Two of the five yeasts studied increased the number of workers in the first few weeks of colony development, suggesting they may improve the micronutrient content of nectar provisions for the first larval generation. Inoculation with each of the five yeasts was also associated with the occurrence of fructo-oligosaccharides in nectar, compounds with known prebiotic effects [89,90]. In a subsequent experiment looking at the effects of yeasts, bacteria, and co-cultures on bumblebee colony development, Pozo *et al.* [88] found that nectar provisions inoculated with bacteria resulted in faster egg-laying and larger brood sizes than control nectar or nectar inoculated with yeasts or co-cultures. Of the eight microbes included across the two studies, microbial effects on bumblebee colony development varied between neutral and positive effects.

(c) Effects of microbe–microbe interactions in nectar on pollinator health

Microbe–microbe interactions within the nectar environment can have consequences for nectar chemistry and pollinator

health. To date, however, the majority of studies have focused on individual taxa [34,36,39]. Nectar microbes frequently engage in competition for resources, often excluding one another via niche preemption or modification [91]. Despite such documented priority effects [20,23,35] and individual flowers being observed to be dominated by individual species [22], surveys of floral nectar have also revealed frequent co-occurrence of microbes (but see [92]). For example, positive associations have been detected between *Metschnikowia* yeasts and *Acinetobacter* bacteria [48]. Co-occurrence between these taxa has been hypothesized to be facilitated through niche partitioning and the assimilation of different nectar resources, as many *Metschnikowia* species are able to ferment glucose, while *Acinetobacter* species can metabolize fructose [93]. This possibility suggests potential for both additive and non-additive effects of nectar microbe assemblages on nectar chemistry and pollinator health. Indeed, recent studies have revealed that yeast and bacteria consortia can increase nectar scent in an additive manner and can have positive effects on honeybee and hoverfly visitation [44,94], yet effects on bumblebee reproduction and colony development are no better than exposure to individual species alone [88]. Considerable work remains, however, as to the effect of nectar microbe consortia on pollinator attraction, nectar feeding, and health [95]. Such consequences may be particularly pronounced when one of the microbes involved is a pathogen.

Flowers are hubs for horizontal transmission of bee pathogens [96,97], both within and among visiting species [98]. As infected individuals visit flowers, they can deposit pathogens via defecation [32,98–100]. Individual bumblebees infected with a pathogen have been found to defecate on flowers more frequently, indicating that transmission at the floral interface may be particularly important for some pathogens of pollinators [32]. Infection of subsequent visitors can occur via consumption of contaminated nectar resources, particularly for faecal-orally transmitted pathogens like the trypanosome *Crithidia bombi* [101]. Although a body of research has accrued to date on the effects of nectar constituents (e.g. secondary metabolites) on pathogen viability and infectivity [102,103], to our knowledge, interactions involving pathogens of pollinators and nectar microbes have received little attention. Circumstantial evidence suggests these interactions can be strong. For instance, a recent *in vitro* experiment revealed that nectar yeasts can suppress the growth of *C. bombi* by upwards of 50%, although the degree of suppression differed among yeast species in the artificial nectar environment tested [36]. The authors hypothesized that the superior competitive ability of the yeasts accounted for this observation. However, additional mechanisms may also be at play. As reviewed above, nectar microbe metabolism can modify sugars, amino acids, secondary metabolites, and pH, factors which have been shown to affect *C. bombi* growth and infectivity [102,104,105]. Many microbe species, including a few that are closely related to common nectar microbes, produce antibiotics [106,107]. Antibiotics, if produced by nectar microbes, may directly affect pathogen viability in floral nectar, resource provisions, or the host gut if they are capable of establishment (but see [36,108]). Going forward, consideration for such interactions within the nectar and host environment could help reveal the transmission of pathogens among wild and managed pollinators.

(d) Indirect effects of non-pollinating floral visitors on pollinator health via nectar microbes

Any visitor that contacts a flower can introduce microbes, in addition to other chemical signatures [109], regardless of its role in pollinating the flower. A diversity of non-pollinating animals visit flowers, from thrips to ants to nectar-robbing bees and birds (figure 2). Each species has its own life history, which can determine the microbes that are vectored by that flower visitor [110]. For example, ants travelling across the forest floor to forage nectar from *Cytinus hypocistis* flowers carry common soil microbes on their body surfaces [31]. Flower visitors that carry little to no pollen may still introduce diverse microbes into flowers. A recent study of a multilevel plant-microbe-pollinator network found that flies (Diptera) and non-bee Hymenoptera are important vectors of microbes despite being poor vectors of pollen [111].

Nectar thieves (figure 2c,d) consume nectar through floral openings without dispersing pollen. Ants are among the most common nectar thieves, collecting nectar from up to 40% of plant species in the tropics and 60% in Mediterranean ecosystems [112,113]. They introduce diverse fungal species to nectar, including basidiomycetes and other common soil microbes [31]. Minute flower-feeding thrips (Thysanoptera; typically ≤ 1 mm) are also common nectar thieves across most terrestrial habitats [114]. Thrips can access unopened flowers and introduce microbes, particularly bacteria, into the nectar environment that can exert priority effects within the nectar microhabitat, thereby excluding subsequently arriving microbes [115].

Unlike nectar thieves, nectar robbers (figure 2e) do not access nectar through floral openings. Instead, primary nectar robbers puncture flowers to extract nectar, bypassing reproductive structures and creating potential routes for external floral epiphytes to enter the nectar [116]. Of the large diversity of primary nectar robbers (reviewed in [116]), only large bees, *Xylocopa californica*, *Bombus bifarius*, and *Bombus mixtus*, have been assessed for their effects on the nectar microbiome [13,117], with distinct nectar microbiomes found to be associated with pollinators and nectar robbers respectively. Flowers robbed by *X. californica* had higher concentrations of monosaccharides and were dominated by an *Acinetobacter* sp. equipped with genes for xylose metabolism, a sugar constituent of wood that is not common in nectar, indicating that this bacterium is adapted to association with carpenter bees [13].

Nectar robbing has been shown to deter pollinators in several flowering species, but the mechanisms behind the deterrence remain unknown despite investigations into visible cues and changes to nectar volumes [118–120]. Microbes vectored by nectar robbers could deter pollinators if they are found in robbed nectar alone, or in higher abundances in robbed nectar. Future studies of pollinator avoidance of robbed flowers should assess the effects of robber-vectored microbes as potential mediators of this interaction. While nectar robbers and pollinators can have distinct effects on the nectar microbiome, it is unclear whether the observed effects of nectar robbers are owing to dispersal of unique microbes by primary robbers, facilitation of microbial dispersal by secondary robbers, or damage to floral tissues. It seems likely that a combination of all three mechanisms results in the distinct nectar microbiomes arising from robber visitation. Future studies should assess the microbial

communities vectored by each visitor species (e.g. microbes on visitor mouthparts) and the effects of the robbing wound on the floral microenvironment (e.g. higher evaporation rates), including consequences for nectar nutritional quality and pollinator health.

The distinct nectar microbes dispersed by non-pollinating floral visitors may have downstream consequences for pollinator health. Further studies of plant-animal-microbe pollination systems at the level of entire flowering communities are needed to clarify the roles of non-pollinating species on nectar microbiomes and the nutritional landscape available to pollinators.

(e) Effects of nectar microbes on lesser-studied pollinators

Many of the examples and hypotheses discussed thus far centre on eusocial bees [36,38–40], and to a lesser degree, hummingbirds [13,34]. However, the health of other invertebrate and vertebrate pollinators (figure 2b) that depend on nectar may also be sensitive to nectar microbes, the chemical changes they induce, and other potential mechanisms discussed. This is especially true given increasing evidence for both the likelihood and frequency for such interactions to occur, as surveys of flowers pollinated by these guilds have revealed that they may harbour abundant and diverse microbial communities [17,92,121].

Among insects, many species in the orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera visit flowers to procure nutritive and non-nutritive rewards. Nectar sugars in particular fuel flight or movement for these insects, especially during periods of dispersal, migration, and congregative behaviours that influence mating and oviposition [122–124]. Studies conducted on individual species across these orders have revealed diverse preferences for nectar sugars. For instance, butterflies and hawkmoths (Lepidoptera) generally prefer sucrose over fructose and glucose in floral nectar [125–128], with this preference being sensitive to concentration differences as low as 5% [129]. Among Dipteran visitors, long-tongued flies are found to be associated with sucrose-dominant nectars [130], while those that are short-tongued or lapping tend to visit hosts with hexose-rich nectars [131,132]. Thus, shifts in nectar sugar profiles stemming from microbial metabolism are likely to affect foraging preferences. However, to our knowledge, little work has been done to address the influence of nectar microbes on non-bee flower visitors. Two studies on Dipteran species have revealed an attraction to nectar yeasts and bacteria: hoverflies and mosquitoes (*Culex pipiens*) are attracted to the bacterium *Acinetobacter nectaris* and nectar yeasts *M. reukaufii* and *Lachancea thermotolerans*, respectively [94,133]. Nectar sugars were not addressed in either of these studies though, with VOCs instead noted to play an important role in attraction. The latter can also probably be said for many angiosperms pollinated by beetles (Coleoptera), whose bouquets are often characterized as being ‘yeasty’ [134–136] with some species having nectar microbiomes dominated by yeasts to match [92]. Careful dissection of the relative contribution of host and microbe to these signals as well as their reliability regarding information on both nectar availability and quality conveyed to visitors should be incorporated into future studies.

Beyond sugars, amino acids in nectar can also influence the attraction and feeding of numerous pollinators

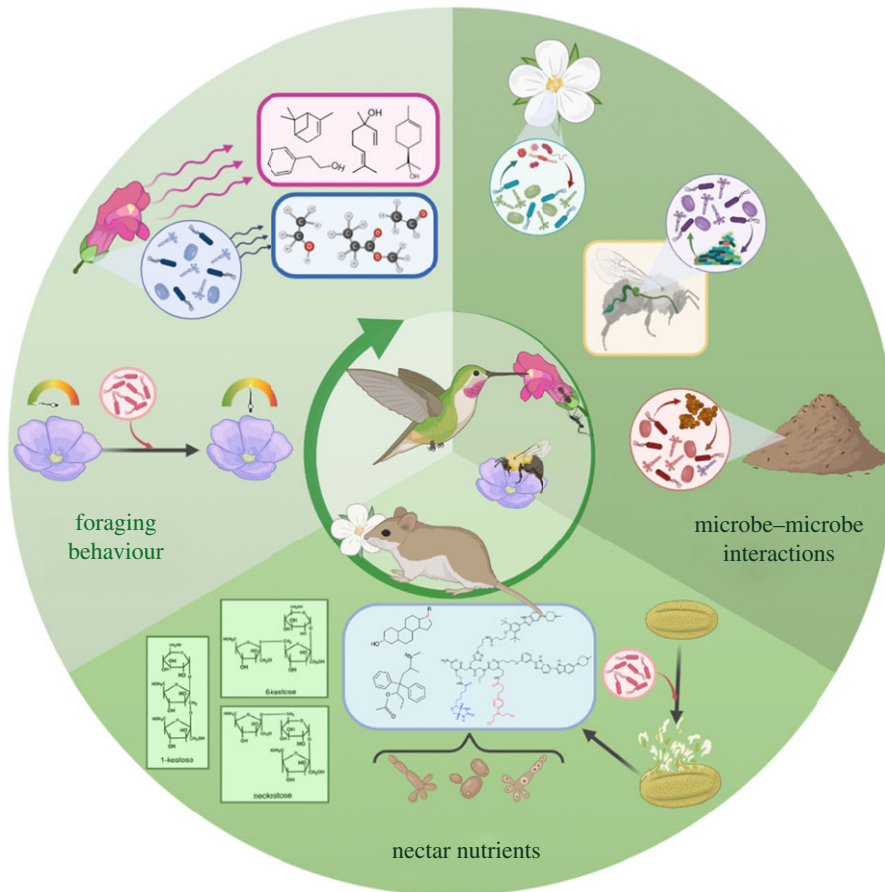


Figure 1. Potential mechanisms by which nectar microbes could affect pollinator health. In this review, the following mechanisms are considered: (i) microbe–microbe interactions in the nectar environment, (ii) microbial impacts on nectar nutritional quality, and (iii) microbial impacts on cues important for pollinator foraging. Figure created with BioRender.com. (Online version in colour.)

[137,138]. This preference for nectar with a higher concentration of amino acids is probably tied to its nutritional value, as their metabolism can influence adult longevity and fitness, as observed in Dipteran and Lepidopteran species [139–141]. As reviewed above, nectar microbes can both increase or decrease amino acid content in nectar, the effects of which are likely to influence the health of pollinators that rely on these resources. Current evidence for such effects is limited and mixed. In two studies conducted with the generalist parasitoid wasp *Aphidius ervi* (Hymenoptera: Braconidae) [37,43], nectar microbe effects were species-dependent, with the bacterium *Lactococcus* sp. enhancing adult longevity and survival, and bacterium *Asaia* sp. and yeasts *Saccharomyces cerevisiae*, *Au. pullulans*, *Sp. roseus*, and *Ha. uvarum* shortening these health metrics. Finally, nectar-specialist yeasts *M. reukaufii* and *M. gruesii* had neutral effects. Though evidence from these studies points to a role for amino acid changes to affect health (e.g. *Lactococcus* sp. increasing content and adult survival), neutral effects of nectar specialist yeasts, despite reducing amino acid content themselves, suggest other factors may also be at play. Additional research is needed, especially for pollinator guilds with life-history stages that may be more sensitive to nectar amino acid content.

Among vertebrate flower visitors, birds are perhaps best known for their role as pollinators, with specialist and generalist species visiting flowering plants for nectar to meet their energetic and nutritional requirements [142–144]. Though a strong dichotomy in nectar traits (volume, concentration and sugar type) has been observed between plants pollinated

by specialist avian nectarivores (e.g. hummingbirds, honeyeaters and sunbirds) and those pollinated by generalists (e.g. bulbuls and starlings) [145], specialist nectarivores can display variable sugar preferences that are concentration-dependent [146–148], suggesting potential for nectar microbe impacts on foraging via sugar metabolism. As with numerous insect visitors [108,115,149,150], some bird pollinators have been found to be effective vectors for yeast dispersal to flowers [17,21]. In one community-wide survey [17], the nectar of bird-pollinated species had a higher incidence and abundance of yeasts than those pollinated by other guilds. Nectar bacteria are also likely to be encountered by specialist and generalist avian nectarivores [151], which may have negative effects on foraging. For instance, hummingbirds (*Calypte anna*) can be averse to the bacterium *Gluconobacter* (*Neokomagataea*) sp. [34], which may stem from its metabolism of nectar sugars and the corresponding secretion of metabolic by-products, namely acetic acid. Understanding links between diet, including exposure to nectar microbes, and avian gut microbiome structure and associated feedbacks on physiology and health are only now starting to receive attention (see [151,152]).

Mammal pollinators are no less important in the maintenance of floral diversity [143], and numerous flying (i.e. bats) and non-flying mammals (e.g. primates, rodents and marsupials) visit flowers to imbibe nectar [2,153]. Mammal-pollinated species are particularly notable for their strong floral scent, which probably facilitates the attraction of such visitors at night when their foraging activity often occurs. These strongly scented volatile bouquets have also often

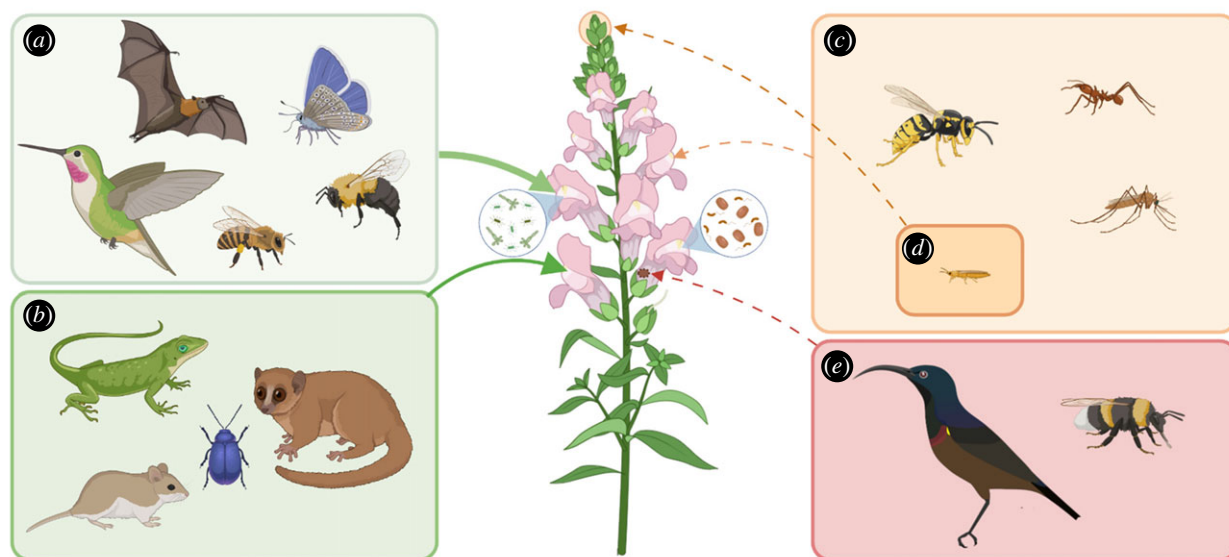


Figure 2. Floral visitor diversity and the nectar microbiome. (a) Common, effective pollinators typically include members of the insect orders Hymenoptera and Lepidoptera, as well as nectarivorous birds and bats. (b) Lesser-studied pollinators include non-flying mammals, reptiles and beetles. (c) Nectar thieves, including (d) thrips, can vector microbes that can then affect pollinator foraging. (e) Nectar robbers can have similar effects. Figure created with BioRender.com. (Online version in colour.)

been described as ‘yeasty’ [154–156], with fermentation-related VOCs hypothesized to be an honest signal for nectar availability [157]. Beyond signalling nectar availability, fermentation by-products may also serve as a nectar feeding stimulant for mammals [158,159]. Such potential has at least been observed in a controlled setting with model laboratory rats, which consume more ethanol when sucrose is added to solution [160]. Furthermore, ethanol catabolism could yield considerable energy gains over that of carbohydrates alone [159]. In nature, mammals such as pen-tailed treeshrews (*Ptilocercus lowii*), slow lorises (*Nycticebus coucang*), and aye-ayes (*Daubentonia madagascariensis*) frequently consume fermented nectar, probably stemming from activity of *Pichia*, *Schizosaccharomyces*, and *Hanseniaspora* yeasts, with alcohol concentrations reaching upwards of 3.8% (v/v) [121,161]. Moreover, a recent preference experiment revealed that slow lorises and aye-ayes can discriminate among nectars that vary in alcohol concentration, even displaying a preference for the highest concentration tested (5%, [161]).

5. Concluding remarks

We have argued that nectar microbes are an integral component of the web of ecological interactions affecting pollinator health.

Knowledge has accumulated in recent years regarding the effects of nectar microbes on nectar chemistry, eusocial bee behaviour, and the establishment of common bee pathogens. In addition, our understanding of pollinator nutritional landscapes and nectar microbe-pathogen interactions is currently expanding. Much work remains to assess the impacts that nectar microbes have on the longevity and reproduction of pollinators. As researchers continue to investigate each of these aspects of pollinator health, conservation and agricultural practitioners will be in a better position to safeguard their populations.

Data accessibility. This article has no additional data.

Authors’ contributions. V.N.M.: conceptualization, investigation, writing—original draft, writing—review and editing; R.N.S.: conceptualization, investigation, writing—original draft, writing—review and editing; T.F.: conceptualization, investigation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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