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Current Opinion in Insect Science

Yeast–nectar interactions: metacommunities and effects on pollinators

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About 90% of all flowering plant species are pollinated by animals. Animals are attracted to flowers because they often provide food in the form of nectar and pollen. While floral nectar is assumed to be initially sterile, it commonly becomes colonized by yeasts after animals have visited the flowers. Although yeast communities in floral nectar appear simple, community assembly depends on a complex interaction between multiple factors. Yeast colonization has a significant effect on the scent of floral nectar, foraging behavior of insects and nectar consumption. Consumption of nectar colonized by yeasts has been shown to improve bee fitness, but effects largely depended on yeast species. Altogether, these results indicate that dispersal, colonization history and nectar chemistry strongly interact and have pronounced effects on yeast metacommunities and, as a result, on bee foraging behavior and fitness. Future research directions to better understand the dynamics of plant–microbe–pollinator interactions are discussed.

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Current Opinion in Insect Science 2021, **44**:35–40

This review comes from a themed issue on **Ecology**

Edited by **Rachel Vannette** and **Robert R Junker**

https://doi.org/10.1016/j.cois.2020.09.014

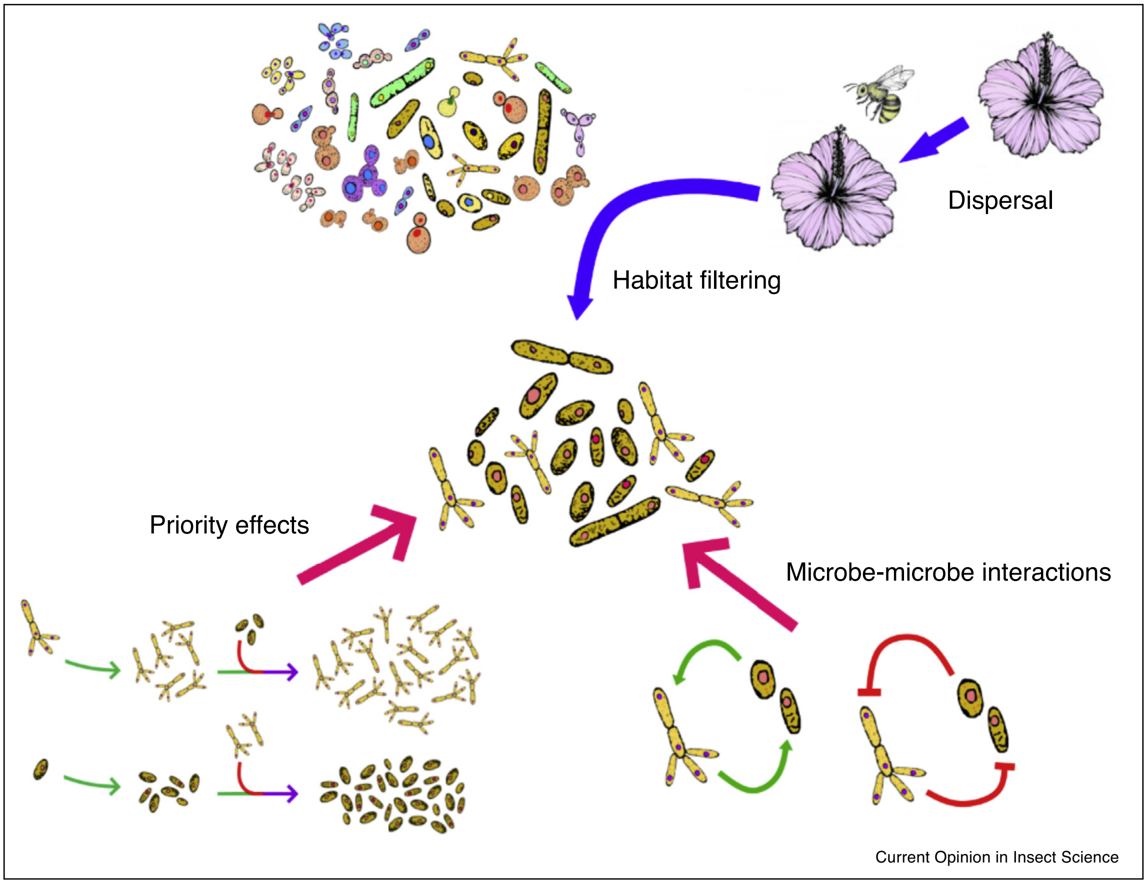
Assembly of nectar yeast communities in floral nectar

About 90% of all flowering plant species are pollinated by animals [1]. In most plant species, insects are the main vectors of pollen, although some plant species are also pollinated by lizards, bats, shrews, birds, or other animals [2]. Insects are attracted to the flowers because they often provide nectar and pollen, which they use to fuel their flight activities and to rear their offspring. Floral nectar mainly consists of water, monosaccharides and disaccharides [3–5] and, although at lower concentrations, amino acids, lipids, minerals, and vitamins [6,7]. In most plant species, floral nectar is dominated by sucrose [8–10], but in some plant species sucrose is hydrolyzed into glucose and fructose by an apoplasmic plant invertase, eventually yielding nectars that consist of a mixture of sucrose and its monomers [5,8]. Besides, traces of other sugars such as arabinose, gentiobiose, lactose, maltose, mannose, melibiose, rhamnose, ribose, stachyose, and trehalose, and the sugar alcohols mannitol and sorbitol have been found in floral nectar [4,8]. Pollen contains mainly proteins, lipids, carbohydrates, and minerals and traces of enzymes, hormones, vitamins, pigments and other minor components [11].

While floral nectar is assumed to be initially sterile, it often becomes rapidly colonized by microorganisms after insects have visited the flowers [12]. Yeasts constitute one of the main inhabitants of nectar. In accordance with their origin, nectar-inhabiting yeasts can be categorized into two distinct groups: (i) yeasts that originate from the atmosphere and usually show no specific adaptations to nectar (high C/N ratio) and pollen (low C/N ratio) conditions, and (ii) yeast species that show much higher levels of specialization and are highly adapted to survive in nectar and pollen [12]. The latter are mainly dispersed from one flower to the next by flower-visiting animals, which in the case of insects may serve as the yeasts' overwintering site when flowers are absent and therefore serve as a transmission vector when flowers become available in early spring [13]. Although yeast communities in floral nectar appear simple [14], recent research has shown that community assembly in this habitat depends on a complex interaction between multiple factors (Figure 1), including the filtering effect of the physical and chemical characteristics of nectar on each yeast species [15], dispersal limitation [16,17], and priority effects caused by history-dependent microbe–microbe interactions [18–21]. Nectar secretion patterns may also affect the assembly of the nectar microbiome [22••].

When individual flowers are viewed as island-like, local microbial habitats [16], the nectar-yeast system constitutes a metacommunity, that is, a regional set of local

Figure 1



Schematic overview of the main factors contributing to the assembly of the yeast communities inhabiting floral nectar. Nectar yeasts are dispersed from flower to flower by insects and other floral visitors. The physicochemical conditions of the nectar environment (e.g. high osmotic pressure, scarcity of nitrogen sources, presence of plant toxins, *etc*.) hinders the growth of some yeast species, thus acting as a filter of the microbial taxa brought by the floral visitors. Additionally, priority effects (i.e. effects that the arrival order and initial abundance of species have on the development of assembling communities at a local site) and microbe–microbe interactions (either positive or negative) may determine the diversity and abundance of yeast species in floral nectar. Interactions with bacteria or other nectar microbes are not represented in this figure, but may also be important drivers of the assembly of yeast communities in nectar that warrant further investigation.

ecological communities that are connected by occasional dispersal among the local communities [23]. The frequency of this microbial dispersal, as affected by pollinator foraging behavior, is therefore a key factor to consider as dispersal frequency can be a major determinant of the persistence and coexistence of yeast species at both local and metacommunity scales [12,13,17].

Impact of nectar yeasts on nectar chemistry

After yeasts have colonized floral nectar, they may change the chemical traits of nectar such as pH and sugar and amino acid content and composition [24•,25], although the effects depend on the yeast species involved. Most yeast species consume glucose and sucrose and decrease amino acid content [9,18,26,27••]. In some cases, nectar yeasts release detectable amounts of byproducts that may increase the fitness of pollinators. For example, Pozo *et al*. [27••] observed a significant increase in the concentration of hetero-oligosaccharides that contained fructosyl–fructose linkages and that were not found in sterile nectar. These molecules may have a prebiotic effect on animals [28]. Nectar also contains plant secondary metabolites that are potentially toxic or deterrent for insects. Microbial activity has been shown to decrease the concentration of these secondary metabolites [29,30]. Apart from inducing changes in sugar and amino acid concentration and the concentration of plant secondary metabolites, yeasts also have the potential to change the odor of floral nectar [31,32]. During sugar fermentation, different volatile organic compounds (VOCs) are released, and sometimes additional compounds are added to the floral olfactory bouquet [33,34••].

Impact of nectar yeasts on foraging behavior

Most pollinators are well equipped to detect and recognize microbial infestation of food sources [35], and yeasts might therefore provide an honest signal (via volatiles) of sugar-rich microsites to plant visitors [36]. Behavioral tests under both controlled greenhouse conditions and field conditions have shown that individual bumblebees generally prefer nectar inoculated with the nectar specialist *Metschnikowia reukaufii* over non-inoculated nectar [37–39]. Although it can be assumed that bumblebees preferentially visit yeast-containing nectar [37,40,41] because they can detect these flowers more easily, or find them more rewarding due to the increased temperature of the nectar [42] or their more complex olfactory display [34••], it remains still unclear what signals the bees are precisely responding to. Recent experiments have also shown that learning of microbial community cues is associative and reward context dependent and mediated by microbial volatiles [43]. At the level of entire colonies, however, no conclusive effects of nectar colonization by yeasts on foraging behavior were found, and effects differed between yeast species [27••]. Addition of *Candida bombiphila* and *Metschnikowia gruessii*, for example, did not increase the probing time of bumblebees foraging on inoculated flowers. Captive bumblebee colonies, however, showed a preference in terms of number of visits for live suspensions of *M. gruessii*, but the opposite was found for *C. bombiphila*.

Impact of nectar yeasts on nectar consumption and pollinator fitness

Because nectar yeasts affect nectar quality and scent, they may also affect pollinator fitness. Altered nectar scent profiles may increase flower visitation rates and therefore nectar consumption, while yeast-induced changes in nectar chemistry and depletion of sugars and amino acids may constitute an energetic cost, especially when yeasts occur at high densities [44]. Experiments using individual bees have shown that the increased attraction of bees to inoculated nectar results in larger amounts of nectar consumption [37,39]. Pozo *et al.* recently investigated the behavior of entire bumblebee colonies that were exposed to artificial nectar solutions with different sugar concentrations and inoculated with different yeast species [27••]. Results showed that bumblebee queens preferred feeders that contained sterile 50% sugar solutions over feeders with 30% sugar water, irrespective of the yeast added to the medium, while workers mostly depleted the treatment feeders with the lowest sugar concentration. These results suggest that differences in feeding preferences were mainly the result of differences in sugar concentrations rather than addition of yeasts themselves. The observed differences in sugar concentration preference between castes are most likely related to the reproductive division of labor in bumblebee colonies. While workers mainly use water and sugar for thermoregulation and foraging, sugars are needed for egg production and therefore may be a more limiting resource for queens than for workers. This may explain why queens were preferentially attracted to sugar feeders with more concentrated sugar water [27••].

Apart from their effects on nectar consumption, yeast cells may also constitute a nutritional supplement for insects as they act as a source of vitamin B, sterols, and minerals [45,46]. Yeast cell digestion by foraging insects may therefore compensate for the costs arising from nutrient depletion and thus provide a fitness benefit, although direct evidence is still largely lacking. Experiments with micro-colonies of *Bombus impatiens* showed that inoculation of nectar by yeasts did not affect the number of worker-laid eggs [39]. Pozo *et al.*, contrastingly, showed that development of entire bumblebee colonies was significantly affected by the presence of yeasts, but the effects depended on the identity of the yeast species added. Interestingly, effects were more pronounced at the colony level than at the individual level, indicating that in the case of social organisms entire colonies should be investigated instead of individual insects to fully grasp the effects of nectar-inhabiting yeasts on pollinator fitness [27••].

Future perspectives

The role of nectar yeasts as insect endosymbionts?

Nectar-inhabiting yeasts have the potential to affect the flower-visiting insects' immune system and health by affecting other microorganisms or microbial products, host products, and food components in the gut of the host insects [46,47]. For example, *in vitro* tests have shown that typical nectar yeasts have the potential to reduce growth of the bee pathogen *Crithidia bombi*, most likely because the pathogen was outcompeted by the superior growing capacities of the yeasts [27••]. Some yeast species have also been shown to facilitate the establishment of a beneficial gut microbiome by releasing non-digestible short-chain sugars that are selectively fermented by beneficial bacteria in the gut [48]. There is some evidence that yeasts added to the diet of bees can temporarily establish in the gut of adult individuals and can even be successfully passed on to future generations [13]. However, at present it remains unclear whether nectar yeasts are able to establish permanently in the gut and how they interact with the resident core microbiota. Future research should, therefore, quantify changes in nectar-inhabiting yeast abundance between food provisions and the gut, and investigate whether nectar-inhabiting yeasts have any importance above and beyond the extra nutrition being added to the food.

Microbial consortia, priority effects, and effects on pollinators

It is clear that addition of individual yeast species to floral nectar can have lasting effects on foraging behavior and fitness of bees. However, there are still very few studies that have investigated the effect of more complex communities consisting of multiple yeast species on nectar quality or pollinator fitness. However, communities that contain more than one microbial species are likely to influence the metabolome differently than monocultures [49,50]. Moreover, floral nectar is not solely colonized by yeasts, but also by bacteria [51–53], which can have markedly different effects on nectar traits [54,55•] and life-history parameters of insects [56]. Because bacteria are incredibly diverse in terms of metabolism, it can be expected that the effects of different taxonomic groups on pollinators will be diverse as well. Currently, little is known about how the relative abundance of bacterial versus yeast groups changes over time in nectar provisions and it is reasonable to assume that, even if they arrive at the same time to floral nectar, yeast and bacteria establish at different times in this habitat. For example, *Pseudomonas* spp. are often the first ones to arrive in floral nectar, with yeasts coming in later once pH and osmotic conditions are conducive. However, depletion of suitable nutrients and production of inhibitory substances by the early colonizers of nectar may hinder the growth of other microbes [20,22••]. In contrast, environmental variability can counteract these inhibitory effects generated by nectar microbes and therefore promote coexistence of yeasts and bacteria in floral nectar [20].

So far, the effects of microbial consortia consisting of a mixture of different yeasts and bacteria on nectar scent, pollinator behavior and fitness have received little attention. A recent study has indicated that volatile emissions of co-cultures that consisted of the yeast *M. reukaufii* and the bacterium *Asaia astilbes* closely resembled the sum of the volatile emissions of the monocultures [57]. Despite these differences in volatile emissions, honey bee feeding did not differ significantly between nectars inoculated with monocultures and co-cultures, suggesting that multispecies assemblages in nectar not necessarily affect foraging behavior and resource consumption by insects. In another study, it was shown that supplementation of bacteria to food provisions of the bumblebee *B. terrestris* led to faster egg-laying, higher brood size and increased production of workers, while supplementation of yeasts or a combination of yeasts and bacteria had less impact on colony development [58]. These results also suggest that yeast–bacteria consortia do not necessarily result in better colony development than the interacting species alone. Besides, VOC profiles and associated insect responses most likely do not only depend on the microbial species added to the nectar, but also on the nutrient medium that is used to cultivate the microorganisms [59]. Most experiments conducted so far used artificial nectars, and it is not unlikely that use of another medium or nectars from different plant species affects scent profiles and as a result the olfactory response of the insects. Future research should, therefore, use different media or natural nectars to investigate how culturing conditions affect the composition of VOC blends and associated response of insects.

Microbial communities in pollen

Apart from nectar, many flower-visiting animals consume pollen, but at present little is known about how microbial presence in pollen affects the chemical properties of pollen, and how these changes affect the foraging behavior and fitness of insects. There is some evidence that microbes can significantly lower the starch level of the pollen [60], but it remains unclear how these changes affect pollinator fitness. Recent research has shown that larvae of mason bees (*Osmia ribifloris*) feeding on increasingly sterile, microbe-deficient pollen provisions experienced significant adverse effects on growth rates, biomass, and survivorship. When larvae were completely deprived of pollen-borne microbes, they consistently exhibited noticeable decline in fitness [61••]. Similarly, administration of microbes through pollen had a stronger positive impact on colony development of *B. terrestris* than when provided via sugar water [49], indicating that microbial colonization of pollen has more pronounced effects on insect fitness than colonization of nectar. In an era of global insect decline [62,63], future research should, therefore, investigate more realistic scenarios that use more complex microbial communities and different nectar sources to better understand the dynamics of plant–microbe–pollinator interactions and the impact of microbes on pollinator behavior and fitness.

Conflict of interest statement

Nothing declared.

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