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SYMPOSIUM ARTICLE

Pollen–Microbe Interactions in Nectar Weakly Influence Bee Foraging Behavior

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Synopsis Plant–pollinator interactions are frequently affected by microbes that grow on flowers. Bacteria and yeast commonly grow within floral nectar, which is a sugar-rich floral reward often sought out by pollinators. Nectar is also commonly contaminated with protein-rich pollen. Microbes can induce this pollen to germinate or burst within the nectar, which potentially results in pollen nutrients being made available to nectar foraging pollinators. Yet whether pollen–microbe interactions in nectar impact pollinator behavior remains unknown. We therefore investigated how a common nectar yeast (*Metschnikowia reukaufii*) and bacteria (*Acinetobacter nectaris*) affected pollen germination and bursting within artificial nectar and effects on bumble bee (*Bombus impatiens*) foraging behavior. We found that both bacteria and yeast reduced the proportion of intact pollen in nectar, with bacteria inducing the most germination and bursting. Although microbes may thus potentially increase the quality of the nectar reward via increased access to pollen nutrients, we did not observe effects on bee flower preference. Similarly, bees did not show increased constancy (i.e., fidelity to one flower type across flower visits) to nectar contaminated with pollen and microbes. In contrast, bees were much more likely to reject flowers with nectar contaminated with pollen and yeast alone or together, relative to flowers that offered uncontaminated nectar. Altogether, our work suggests pollen–microbe interactions within nectar may have relatively minor influences on pollinator foraging behavior. We discuss possible explanations and implications of these results for plant and pollinator ecology.

Introduction

Pollinator foraging decisions are frequently influenced by the composition of floral rewards (e.g., nectar and pollen) (Fenster 1991; Leonard et al. 2011; Fowler et al. 2016; Nicolson 2022). For instance, nectar is composed primarily of sugars, as well as micronutrients and minor metabolites (Nicolson 2022), and bees often prefer nectar supplemented with amino acids (Alm et al. 1990; Hendriksma et al. 2014). Floral nectar also provides an ideal environment for the growth of yeast and bacteria (Brysch-Herzberg 2004; Fridman et al. 2011), which are common and abundant members of nectar microbial communities (Herrera et al. 2008, 2009b). These microbes are highly sensitive to the content of floral nectar, with sugars, amino acids, and antimicrobial compounds in the nectar significantly affecting microbial growth (Brysch-Herzberg 2004; Herrera et al. 2009a; Lievens et al. 2015; Christensen et al. 2021; Schmitt et al. 2021). In turn, microbial communities in flower nectar can influence pollinator foraging behavior, as changes in nectar chemistry mediated by yeast and bacteria often alter the sensory cues available to pollinators, such as nectar scent and taste (Rering et al. 2017; Russell and Ashman 2019). In addition to microbes, nectar chemistry might also be affected by pollen, which frequently falls into the nectar as a result of wind or pollinator activity (Jones and Jones 2001; Herrera 2017). Unlike nectar, pollen contains primarily proteins, starches, and lipids (Stanley and Linskens 1974; Thakur and Nanda 2020), and thus pollen present in nectar could alter the nutrients available to both nectar-foraging pollinators and nectar microbes (Christensen et al. 2021).

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Regardless, how pollen–microbe interactions in nectar subsequently affect pollinator foraging decisions have yet to be examined.

Although pollen is often found within nectar (Herrera 2017), pollen nutrients may not be accessible to nectar-foraging pollinators or nectar-inhabiting microbes, because the nutrients are encased within the extremely durable outer exine wall of the pollen (composed of the biopolymer, sporopollenin; Roulston and Cane 2000; Wang and Dobritsa 2018). Yet there is growing evidence that nectar microbes can break down the pollen exine or trigger pollen germination, leading to the release of pollen's nutrient-rich cytoplasmic contents (Eiskowitch et al. 1990; Christensen et al. 2021; Crowley and Russell 2021). Consequently, we expect that when both pollen and microbes co-occur in nectar, pollinators may prefer this nectar due to the supplementary nutrients released from the pollen, and that pollinators would also be less likely to switch to an alternate flower type lacking these nutrients. Conversely, in the absence of nectar microbes, we should expect pollinators to show no preference for nectar contaminated with pollen and to switch more frequently among equivalent flower types, assuming that pollen nutrients are generally inaccessible in the absence of nectar microbes.

In addition, nectar microbes often directly influence pollinator decisions (Herrera et al. 2013; Schaeffer et al. 2017). For example, nectar-inhabiting bacteria and yeasts can produce volatile compounds that modify the attractiveness of nectar to pollinators (Vannette and Fukami 2016; Rering et al. 2017). Microbial effects on nectar preference can also differ among microbial taxa. For instance, bacteria in the genus *Gluconobacter* decrease nectar sugar concentration and alter its pH, often reducing pollinator visitation (Vannette et al. 2013). In contrast, yeasts like *Metschnikowia reukaufii* often ferment sugars and produce volatiles that sometimes facilitate pollinator visitation (Rering et al. 2017). Microbial taxa may also differ in their capacity to release pollen nutrients into the nectar. For instance, some bacteria are thought to germinate pollen readily, whereas some yeast suppress germination (Eiskowitch et al. 1990; Christensen et al. 2021). Consequently, the interaction between how microbes affect nectar quality, and the release of pollen nutrients into nectar by microbes could lead to complex outcomes for pollinator behavior. For instance, pollinators often reject flowers with nectar that contain bacteria (e.g., Vannette et al. 2013; Good et al. 2014). However, when nectar is contaminated with pollen and bacteria, this nectar might no longer be distasteful and might even be preferred, due to the now readily accessible pollen nutrients. Similarly, if yeast do not readily release pollen nutrients into nectar, pollinators may show a preference for yeast-inoculated nectar (e.g., Vannette et al. 2013; Rering et al. 2017), but no increased preference for nectar that is also contaminated with pollen.

In this laboratory study, we first assessed how readily common nectar bacteria and yeast induced pollen to germinate or burst in artificial nectar. We hypothesized that consistent with prior studies, bacteria (*Acinetobacter nectaris*) would induce pollen germination/bursting more readily than yeast (*Metschnikowia reukaufii*), and that most pollen would remain intact in sterile nectar. We next assessed how an ecologically realistic quantity of pollen or nectar microbes alone or together in artificial nectar affected bumble bee (*Bombus impatiens*) foraging behavior. We hypothesized that due to the release of pollen nutrients by microbes, bees would strongly prefer nectar contaminated with pollen and microbes together and have no preference for nectar contaminated with only pollen. We also hypothesized that bees would show greater floral constancy to artificial flowers with nectar contaminated with pollen and microbes together, due to the presumably higher quality nectar. Finally, for the same reason, we predicted that bees would be less likely to reject the nectar of artificial flowers contaminated with pollen and microbes together.

Materials and methods

Experimental subjects

To study how pollen–microbe interactions in nectar affected bumble bee behavior, we used 90 flower-naïve workers from lab-reared common eastern bumble bees (*Bombus impatiens*) from three commercially obtained colonies (Plant Products, Canton, MI, USA). Following Russell et al. (2017), each colony was maintained on 20% sucrose solution by w/w from artificial feeders within enclosed foraging arenas (LWH: 82 × 60 × 60 cm) set to a 14 h:10 h light:dark cycle. Pulverized honeybee-collected pollen (Koppert Biological Systems) was deposited directly within the colony boxes for bees to forage on.

We cultured two kinds of microbes commonly isolated from bees and flowers (Brysch-Herzberg 2004; McFrederick et al. 2012; Pozo et al. 2012; McFrederick et al. 2018) and that have previously been found to interact with pollen in nectar (Eisikowitch et al. 1990; Christensen et al. 2021): a yeast, *Metschnikowia reukaufii* (isolated from *Epilobium angustifolium* nectar), and a bacterium, *Acinetobacter nectaris* (isolated from *Streptanthus tortuosus* nectar). To grow yeast we used sterile YM + 2% fructose (in 1 L water: 3 g each yeast and malt extracts, 5 g peptone, 10 g glucose) and to grow bacteria we used R-2A broth (Himedia

Laboratories, PA, USA). Both microbes were grown in a shaker for 48 hours at 30°C and 260 rpm, from parent stock stored at −80°C in 25% glycerol. We created a growth curve for each strain by counting microbes via hemocytometer at 400× (ACCU-SCOPE 3000-LED microscope, Commack, NY, USA) at progressively greater optical densities, measured via a spectrophotometer (Thermo Spectronic 20D + Spectrophotometer, Waltham, MA, USA). We used these growth curves to determine how dilute or concentrated samples needed to be made to achieve ecologically realistic cell densities of yeast and bacteria (up to 10^5 yeast cells and 10^7 bacterial cells per μL in floral nectar; Alvarez-Perez et al. 2019). Strains were thereafter transferred to separate 1.5 mL sterile microcentrifuge tubes and put through 2 rounds of centrifuging (10,000 rpm for 5 min) and sterile saline (8.5% NaCl) substitution and elimination to purge media. Strains were stored at 4°C for up to 5 days for behavioral trials and regrown weekly from the parent stock.

Pollen germination and bursting experiment

To assess whether yeast and/or bacteria influenced pollen germination and bursting, we first prepared three kinds of sterile artificial nectar solutions (40% sucrose solution by w/w) into which we added approximately 116 live pollen grains per μL (commercially available cherry pollen, *Prunus avium*; Pollen Collection and Sales; Lemon Cove, CA, USA), the mean pollen concentration across 91 flowering plant species (see Herrera 2017). Cherry flowers produce both nectar and pollen simultaneously, making pollen–microbe interactions within the nectar likely. Additionally, bumble bees readily collect cherry pollen (Russell et al. 2017) and thus nutrients from cherry pollen might also enhance nectar quality. To the pollen–nectar solutions we then added yeast (4×10^4 yeast cells per μL), bacteria (1.2×10^6 bacterial cells per μL), or sterile nectar (as a control), vortexed the solutions, and incubated them at room temperature (15 replicates per solution type). After 60 min, 10 μL aliquots from each solution were examined at 400× and the number of intact, germinated, and burst pollen grains were counted via hemocytometer. We chose this timeframe based on findings by Christensen et al. (2021), which showed high pollen germination rates between 45 and 90 min. Pollen was considered to have germinated if a pollen tube was visibly protruding from the exine; a pollen grain was considered to have burst if protoplasm was discharged from the exine or if the exine was in pieces (following Christensen et al. 2021).

Behavioral experiment

To identify appropriate test subjects and accustom bees to foraging for nectar on a 6 × 5 horizontal array of equal numbers of sterile purple and blue plastic artificial flowers, we conducted training trials in which groups of bees were allowed to forage on an array of 6 × 5 white plastic flowers (each flower contained 10 μL of 40% sucrose in its nectary). We captured bees that foraged, marking them uniquely with non-toxic oil markers (Sharpie, CA, USA), and returned them to their colonies.

We divided marked flower-naïve bees into two experimental groups, each with three treatments; each treatment had two sub-treatments (Fig. 1). A maximum of three colonies were represented per sub-treatment. Across all trials we set up a 6 × 5 horizontal array of equal numbers of sterile blue and purple colored plastic flowers on the test arena floor. Each flower received an artificial nectary (a sterile 1.5 μL microcentrifuge cap), into which 3 μL of artificial nectar solution was added into the center of the cap. For one experimental group, the three treatments differed in terms of whether one of the two flower colors offered nectar inoculated with pollen, yeast, or pollen plus yeast; or in the other experiment: pollen, bacteria, or bacteria plus pollen (Fig. 1). The sub-treatments differed in terms of which flower color offered sterile nectar, which was systematically alternated among trials (Fig. 1). We used two flower colors in each horizontal array to facilitate bees being able to choose between the nectar solution types (i.e., by associating a flower color with a specific type of nectar solution).

To initiate a behavioral trial, flowers were set up and a single marked worker bee was gently captured from the foraging arena using a 40 dram vial (Bioquip, CA, USA) and immediately released in the center of the test arena following Russell et al. (2017). We recorded when the bee landed on a flower (touching the flower with at least 3 of its legs simultaneously) and extended its proboscis into the nectary for more than 1 second ("drinking"), and when the bee landed on a flower and either did not drink or extended its proboscis into the nectary only briefly (<<1 second) ("rejections"). Immediately after each visit that involved drinking, we refilled the artificial nectary with the appropriate nectar solution, such that flowers were never depleted. To ensure trials were comparable, we terminated a trial after the bee reached 40 visits to flowers or, rarely, if the bee did not approach any flower for a period of 5 min, whichever came first. To terminate a trial, we turned off the

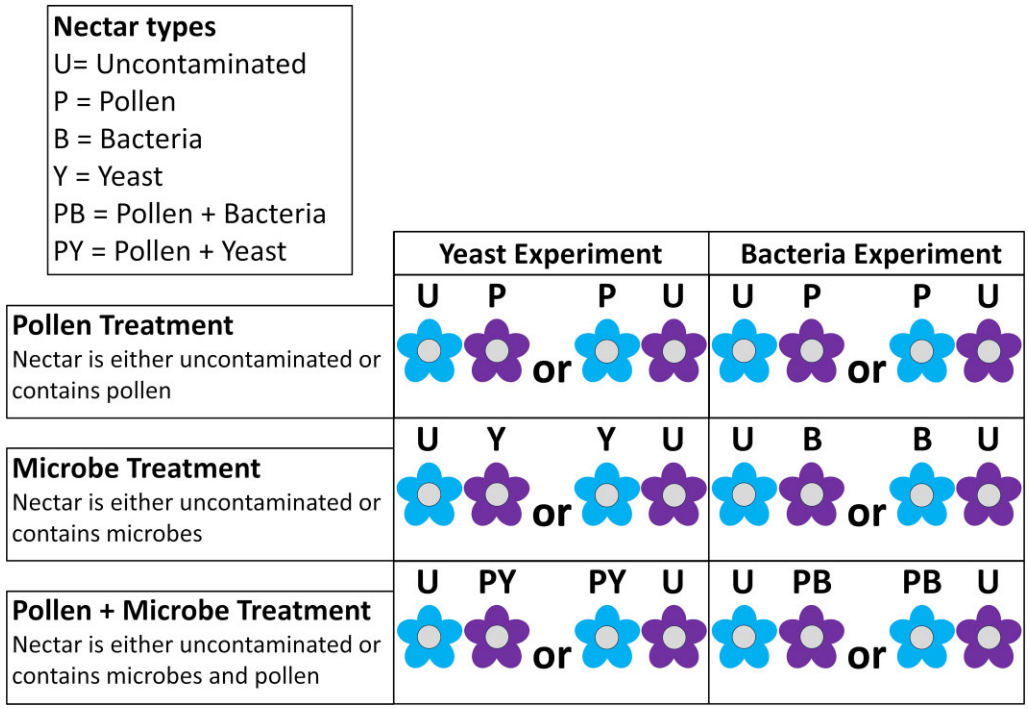

Fig. 1 Schematic of the two experiments. In the yeast experiment, bees were split into three treatments and an individual was allowed to forage on flowers that either had (pollen treatment) uncontaminated nectar or nectar contaminated with pollen; (yeast treatment) uncontaminated nectar or nectar contaminated with yeast; (yeast and pollen treatment) uncontaminated nectar or nectar contaminated with yeast and pollen. In the bacteria experiment, bees were split into three treatments and an individual was allowed to forage on flowers that either had (pollen treatment) uncontaminated nectar or nectar contaminated with pollen; (bacteria treatment) uncontaminated nectar or nectar contaminated with bacteria; (bacteria and pollen treatment) uncontaminated nectar or nectar contaminated with bacteria and pollen. Sub-treatments differed in terms of which flower color offered which nectar type.

overhead arena lights and captured the bee in a vial. After a bee completed its trial, it was euthanized to prevent it from returning contaminated nectar to its colony. After each trial, artificial nectaries were soaked in 70% ethanol for at least 30 min and then rinsed repeatedly in sterile water and allowed to air dry before being reused.

Data analysis

All data were analyzed using R v.4.4.0 (R Development Core Team 2024). We checked model assumptions for all following models using DHARMa (DHARMa package; Hartig 2022). We also conducted visual diagnostics with sjPlot (Lüdecke 2023).

Do nectar bacteria and yeast induce germination/bursting of pollen?

To verify that yeast and bacteria affect pollen germination and bursting and therefore could potentially release pollen contents that might influence bee preference, we performed an ANOVA in base R on the percentage of intact pollen grains across the three nectar treatments ("pollen control," "pollen + yeast contamination," and "pollen + bacteria contamination"). We then ran Tukey's post hoc test in base R to determine which pairs were significant.

Do microbes or pollen alone or together in nectar affect bee preference?

Next, we investigated whether bumble bees demonstrated a preference for nectar containing microbes alone or in combination with pollen by using generalized linear mixed effects models (GLMMs) with a binomial distribution using the glmmTMB() function (glmmTMB package; Brooks et al. 2017), specifying type II Wald chi-squared ($\chi 2$)-tests via the Anova() function (car package; Fox and Weisberg 2019). We used a separate GLMM for each experimental group ("yeast," "bacteria"). For each GLMM, the response variable was flower landing preference (the flower type for flower visits that involved drinking: contaminated vs uncontaminated) and the explanatory variables were treatment ("microbe contamination," "pollen contamination," "pollen + microbe contamination") and experience ("visit number"). We included "days since nectar

prepared" and "bee ID" as random factors, with "visit number" as repeated measures within bee ID. Models would not converge with colony ID as a random factor. In cases of significant effects, we ran Tukey's post hoc test using the emmeans() function (emmeans package; Lenth 2024) to determine which pairs were significant.

Do microbes or pollen alone or together in nectar affect the frequency of flower switching?

To assess if the presence of microbes alone or in combination with pollen influenced the frequency with which bees switched between the two flower types in each trial described above, we again used two GLMMs, one for each experimental group ("yeast," "bacteria"). For each GLMM, the response variable was flower switching ("switch" vs "no switch": a switch was classified as the bee shifting the flower type it drank from between consecutive visits) and the explanatory variables were "treatment" and experience, as above. We also specified random effects as above.

Are bees more likely to reject flowers when nectar contains microbes or pollen alone or together?

Additionally, we examined whether nectar containing microbes alone or in combination with pollen affected the proportion of rejections on each flower type (landing without drinking). We again used GLMMs as above, and the response variable for each GLMM (one for each experimental group) was proportion rejections (for both flower types for each behavioral trial) and the explanatory variables were "treatment" and "flower type" (either "uncontaminated" or "contaminated"). Random effects were specified as "days since nectar prepared" and "bee ID" within "colony ID." To meet model assumptions, we added 0.01 to each response variable and square root transformed them.

Results

Nectar bacteria and yeast induce germination/bursting of pollen

The percentage of pollen grains that were intact (no visible pollen tubes or broken exine) depended significantly on whether the pollen was added to sterile nectar, nectar inoculated with yeast (*M. reukaufii*), or nectar inoculated with bacteria (*A. nectaris*) (Fig. 1; ANOVA: $F_{2,42} = 67.67$, $P < 0.001$). While on average most (74%) pollen grains added to sterile nectar remained intact, only 56% and 29% of pollen in nectar inoculated with yeast and bacteria, respectively, remained intact (Fig. 2).

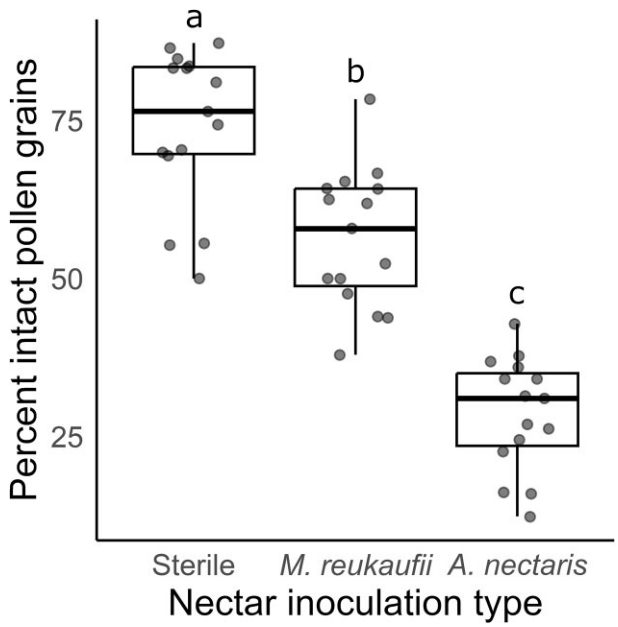

Fig. 2 Effect of inoculation treatment (no inoculation "control," yeast "*M. reukaufii*," and bacteria "*A. nectaris*") on the percentage of intact pollen grains in artificial nectar (40% sucrose by w/w). $N = 15$ replicates assessed per treatment. Plotted as boxplots with the individual data points superimposed. Different letters above boxplots indicate significant differences among treatments at $P < 0.05$.

Microbes or pollen alone or together in nectar do not affect bee preference

Given that intact pollen grains are less common in yeast and bacteria-inoculated nectar and thus may release pollen contents within the nectar, we examined whether bumble bees preferred pollen and microbes together or alone in nectar versus uncontaminated nectar. We found no significant preference for nectar with pollen and/or microbes (yeast or bacteria) and bees did not modify their preference with experience (Fig. 3A; GLMM: yeast contamination experiment: effect of treatment: $\chi^2_2 = 0.541$, $P = 0.763$; effect of experience: $\chi^2_1 = 0.015$, $P = 0.903$; effect of treatment × experience: $\chi^2_2 = 1.063$, $P = 0.589$; Fig. 3B; GLMM: bacteria contamination experiment: effect of treatment: $\chi^2_2 = 0.187$, $P = 0.911$; effect of experience: $\chi^2_1 = 0.006$, $P = 0.941$; effect of treatment × experience: $\chi^2_2 = 2.714$, $P = 0.257$).

Bacteria and yeast did not affect the frequency of flower switching

In neither experiment did pollen or microbes (yeast or bacteria) together or alone in nectar significantly affect the frequency of flower switching (Fig. 4; GLMMs: effect of treatment; yeast experiment: $\chi^2_2 = 1.511$, $P = 0.470$; bacteria experiment: $\chi^2_2 = 2.726$, $P = 0.256$).

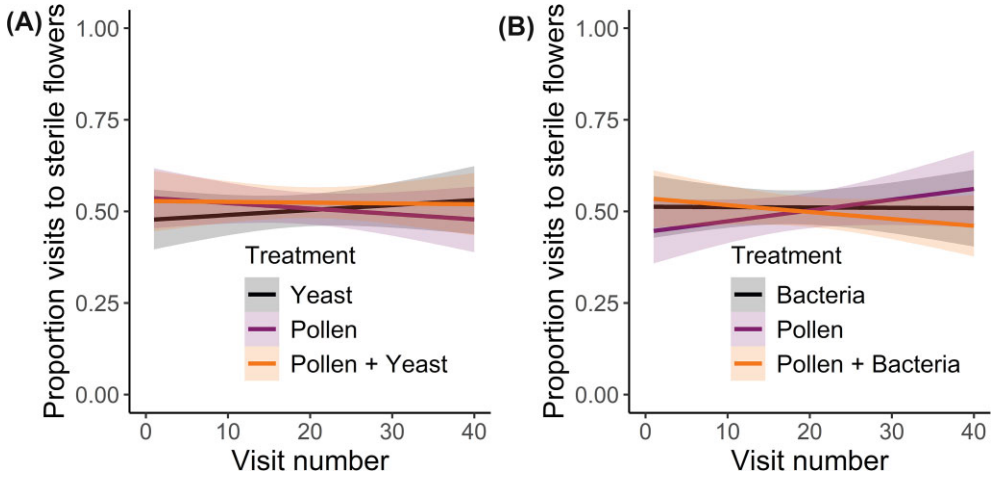

Fig. 3 Proportion of visits to flowers with uncontaminated nectar versus flowers whose nectar was contaminated with (A) pollen, yeast (*M. reukaufii*), or with pollen and yeast together or (B) with pollen, bacteria (*A. nectaris*), or with pollen and bacteria together. For the yeast contamination experiment, $N = 15$ bees each for the pollen, yeast, and pollen with yeast treatments. For the bacteria contamination experiment, $N = 13$, 15, and 17 bees for the pollen, bacteria, and pollen with bacteria treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% confidence intervals.

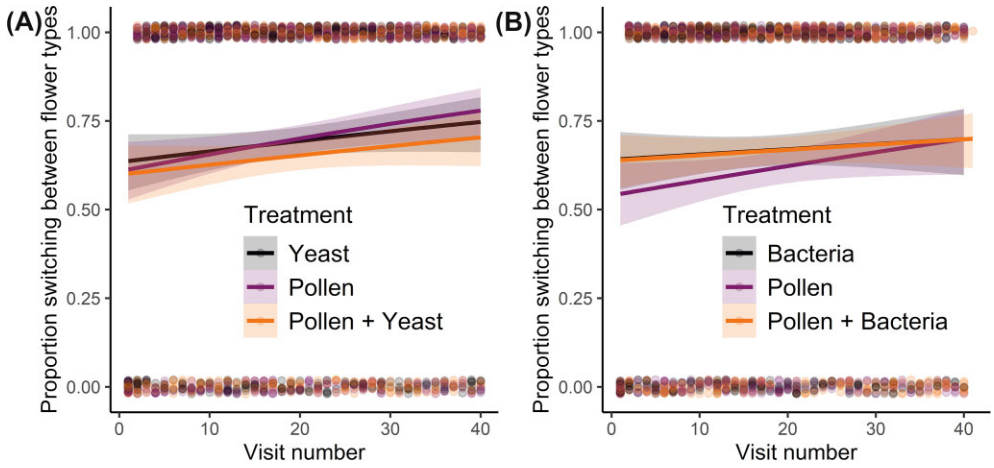

Fig. 4 The frequency of switching between flowers with uncontaminated nectar versus flowers whose nectar was contaminated with (A) pollen, yeast (*M. reukaufii*), or with pollen and yeast together or (B) with pollen, bacteria (*A. nectaris*), or with pollen and bacteria together (same dataset as in Fig. 3, analyzed for different sampling behavior). For the yeast contamination experiment, $N = 15$ bees each for the pollen, yeast, and pollen with yeast treatments. For the bacteria contamination experiment, $N = 13$, 15, and 17 bees for the pollen, bacteria, and pollen with bacteria treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% confidence intervals.

Instead, bees in both experiments switched among flower types more frequently with experience (Fig. 4A; GLMMs: yeast experiment: effect of experience: $\chi^2_1 = 10.134$, $P = 0.001$; effect of treatment × experience: $\chi^2_2 = 0.794$, $P = 0.672$; bacteria experiment: effect of experience: $\chi^2_1 = 4.046$, $P = 0.044$; effect of treatment × experience: $\chi^2_2 = 0.855$, $P = 0.652$).

Bees reject contaminated nectar more frequently in the yeast contamination experiment than uncontaminated nectar

In the yeast contamination experiment, bees rejected a greater proportion of flowers with pollen and/or yeast in the nectar than they did flowers with uncontaminated nectar (38% greater) (Fig. 5; GLMM: effect of flower type: $\chi^2_1 = 4.527$, $P = 0.033$). However,

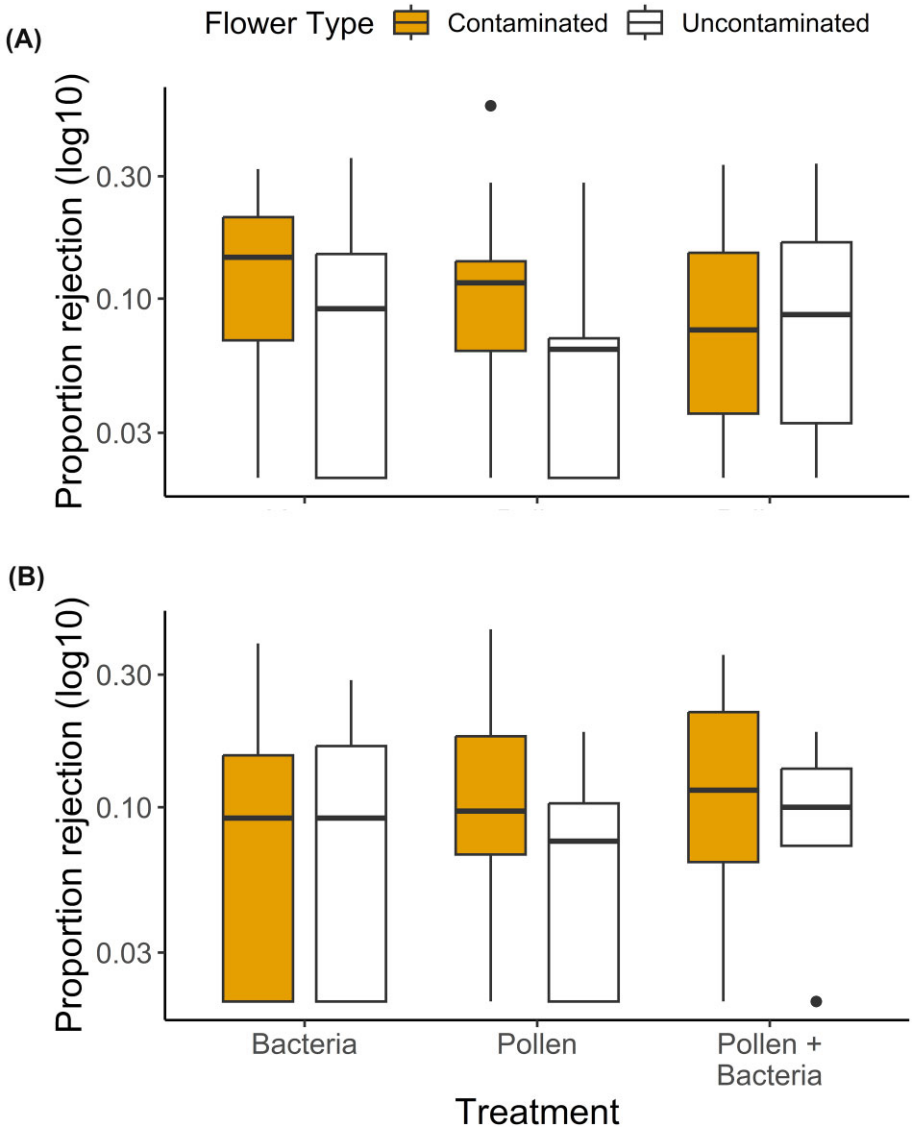

Fig. 5 The proportion of flowers rejected (landing without drinking) with uncontaminated nectar versus flowers whose nectar was contaminated with (A) pollen, yeast (*M. reukaufii*), or pollen and yeast together or (B) pollen, bacteria (*A. nectaris*), or pollen and bacteria together (same dataset as in Fig. 3, analyzed for different sampling behavior). Plotted as boxplots, representing the variance across bees. For the yeast contamination experiment, $N = 15$ bees each for the pollen, yeast, and pollen with yeast treatments. For the bacteria contamination experiment, $N = 13$, 15, and 17 bees for the pollen, bacteria, and pollen with bacteria treatments, respectively.

neither treatment nor the interaction between treatment and flower type significantly affected how frequently bees rejected flowers (GLMM: effect of treatment: $\chi^2_2 = 0.759$, $P = 0.684$; treatment × flower type interaction: $\chi^2_2 = 3.498$, $P = 0.174$). In the bacterial contamination experiment, the proportion of rejected flowers did not depend on treatment, flower type, or their interaction (Fig. 5; GLMM: effect of treatment: $\chi^2_2 = 0.217$, $P = 0.897$; effect of flower type: $\chi^2_1 = 2.575$, $P = 0.109$; treatment × flower type interaction: $\chi^2_2 = 1.603$, $P = 0.449$).

Discussion

Our study sheds light on how pollen–microbe interactions in nectar may influence the behavior of nectar foraging bees. Consistent with Christensen et al. (2021), we found that a bacteria (*A. nectaris*) can rapidly induce pollen germination and bursting within nectar. Additionally, we found that a yeast (*M. reukaufii*) can also induce germination and bursting, although to a lesser extent. However, although we used concentrations and types of yeast, bacteria, and pollen commonly found

in nectar (Herrera 2017; Alvarez-Perez et al. 2019), we observed only modest effects on bumble bee behavior. Most surprisingly, despite enhanced pollen germination and bursting in nectar inoculated with microbes (resulting in presumably more nutrient-rich nectar), bees did not exhibit a preference for this nectar. Similarly, although we expected bees to switch less when nectar was presumably of higher quality (i.e., contaminated with microbes and pollen), this was not the case, and bees actually switched more frequently among flowers with uncontaminated and contaminated nectar as they gained experience. Altogether, our results indicate that bees generally assessed nectar contaminated with pollen and microbes as equivalent to uncontaminated nectar and suggest that pollen nutrients released by nectar microbes may not have significantly enhanced the quality of the nectar reward.

Assuming yeast and bacteria made pollen nutrients (e.g., amino acids, proteins, and lipids) freely available in nectar, why were effects on pollinator behavior so limited? One possibility is that nutrient concentrations were either too low or too high to elicit strong responses. For instance, bumble bees can detect amino acid concentrations in nectar and may respond neutrally or aversively depending on the concentration of the particular amino acid (Inouye and Waller 1984; Reynolds and Leonard 2015). Although we used the mean concentration of pollen reported in the literature for nectar (Herrera 2017), it is possible that behavioral responses are only prominent at lower or higher concentrations (which could vary among pollen types depending on their nutrients). Furthermore, the microbes themselves may have reduced the concentration of pollen nutrients to below what bees typically respond to. For example, Christensen et al. (2021) found that bacteria release and use protein from pollen as a nitrogen source to grow to high density in nectar, although yeast did not. Although we did not characterize microbial growth or protein release in nectar, flower preference and switching behavior did not depend on whether pollen was inoculated with yeast or bacteria, perhaps suggesting that both kinds of microbes reduced freely available pollen nutrients. However, we also did not observe differences in bee behavior coinciding with time after nectar was inoculated (data available in supplementary material), suggesting that nutrient release and increasing use by microbes alone were insufficient to influence preference or switching.

Our results are perhaps less surprising when put in context of the many studies examining pollinator preference for endogenous nectar amino acids and proteins. Nectar uncontaminated with pollen nearly ubiquitously contains these compounds at low concentrations (Gottsberger et al. 1984; Petanidou et al. 2006; Göttlinger and Lohaus 2023), but effects on pollinator behavior appear to be highly variable. For instance, types of amino acids in nectar differ in whether they attract (e.g., Petanidou et al. 2006; Bertazzini et al. 2010), deter (e.g., Petanidou et al. 2006; Bertazzini et al. 2010; Hendriksma et al. 2014; Villagómez et al. 2024), or have no effect on pollinator preference (e.g., Roubik et al. 1995; Hendriksma et al. 2014). It is thus possible that the reason we did not observe effects of pollen–microbe interactions on pollinator preference was due to the particular types and/or concentrations of compounds released into artificial nectar from the cherry pollen that we used. Additionally or alternatively, the influence of pollen compounds on pollinator behavior may depend on nectar sugar concentration. For example, at moderate nectar sugar concentrations, phenolic acids (which occur in nectar and in pollen) serve as attractants; however, at low or high nectar sugar concentrations, these acids have a deterrent effect (Liu et al. 2006). Future work will be required to determine whether the effect of pollen–microbe interactions on pollinator behavior depends on nectar sugar concentration and pollen type. Finally, even amino acids in nectar that do not strongly influence initial preference can be learned associatively by pollinators (e.g., Kim and Smith 2000; Simcock et al. 2014; Broadhead and Raguso 2021). Thus, future studies should examine whether pollen–microbe interactions in nectar influence associative learning, a powerful mechanism driving foraging behavior and floral trait evolution (Schiestl and Johnson 2013).

Nectar yeast and bacteria are frequently found to affect the preference of pollinators, including of bumble bees (e.g., Herrera et al 2013; Junker et al. 2014; Lindblom 2012; Rering et al 2017; Russell and McFrederick 2021). Additionally, nectar is colonized by diverse bacteria and fungi, but nectar of individual flowers is often dominated by relatively few bacterial and fungal strains (e.g., Herrera et al. 2009a; Álvarez-Pérez et al. 2024). Yet when yeast or bacteria were alone in nectar, we observed no effect on bee preference or switching behavior. Although behavioral responses can depend on the density of microbes in nectar (Junker et al. 2014), we used ecologically realistic concentrations previously found to elicit pollinator responses (e.g., Junker et al. 2014; Rering et al 2017; Yang et al. 2019). Perhaps, however, pollinators respond differently to nectar microbes depending on nectar sugar concentration. While sugar concentration of nectar varies considerably (5% to 70% for live flowers; Pattrick et al. 2024), sugar-rich nectars are preferred by bees (Zhou et al. 2024). Compared to prior studies, we used artificial

nectar with a higher sugar concentration (40%), which, while within the natural range for bee-pollinated flowers (Nicolson et al. 2007; Pamminger et al. 2018), would have been more preferred and thus may have masked the effect of microbial contamination to some degree. Consistent with this, although bumble bees were more likely to reject nectar with yeast and pollen (suggesting they could detect the contamination to some degree), their overall preference and switching behavior was unaffected. Interestingly, bees were not more likely to reject nectar with bacteria, in contrast to prior studies showing generally deterrent effects of nectar bacteria (Good et al. 2014; Junker et al. 2014; Rering et al 2017).

Our findings raise important questions about the ecological impact of pollen–microbe interactions in nectar. Pollen and microbial contamination of flower nectar is extremely common (Herrera et al. 2013; Alvarez-Perez et al. 2019; Martin et al. 2022) and consequences of these interactions for plant health and fitness have not been explored. Even assuming that pollinator behavior is generally unaffected (but see earlier discussion of potential context-dependency), accelerating the growth of certain nectar microbes could benefit the plant if this reduced the potential for pathogenic microbes to invade the nectary (e.g., via priority effects; Rering et al. 2023). In contrast, facilitating nectar microbial growth might harm the plant if this typically increased rates of flower senescence (e.g., Vannette and Fukami 2018). Likewise, availability of pollen nutrients could influence cross-feeding and microbial community assembly dynamics (Dhami et al. 2016; Mueller et al. 2023), which in turn could influence cues used by pollinators (e.g., Russell and Ashman 2019). Finally, nectar of live flowers is much more nutritionally complex than simple sucrose solution used in laboratory behavioral trials (Nicolson 2022; Liu et al. 2006), and effects of nectar microbes on natural nectar are often complex and involve the addition and alteration of nutrients (Vannette and Fukami 2018; Herrera and Alonso 2025). Consequently, pollen–microbe interactions in natural nectar might be more likely to influence pollinator behavior than our laboratory results would suggest.

Author contributions

S.C.E. and A.L.R. conceived and designed the experiments. S.C.E., C.C.D., M.V.R., L.P.C., and A.L.R. performed the experiments and collected the data. S.C.E. and A.L.R. analyzed the data. S.C.E. and A.L.R. wrote the original draft of the manuscript; other authors provided editorial advice.

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Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

None declared.

Data availability

The datasets supporting this article are available as electronic supplementary material.

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