BIOLOGY LETTERS

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Cite this article: Schaeffer RN, Rering CC, Maalouf I, Beck JJ, Vannette RL. 2019 Microbial metabolites elicit distinct olfactory and gustatory preferences in bumblebees. *Biol. Lett.* **15**: 20190132.
http://dx.doi.org/10.1098/rsbl.2019.0132

Received: 27 February 2019
Accepted: 17 June 2019

Subject Areas:
behaviour, cognition, ecology, plant science, evolution

Keywords:
Asaia astilbes, bumblebee, *Metschnikowia reukaufii*, microbial volatile organic compounds, nectar microbes, pollination

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4560635.

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Animal behaviour

Microbial metabolites elicit distinct olfactory and gustatory preferences in bumblebees

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Animals such as bumblebees use chemosensory cues to both locate and evaluate essential resources. Increasingly, it is recognized that microbes can alter the quality of foraged resources and produce metabolites that may act as foraging cues. The distinct nature of these chemosensory cues however and their use in animal foraging remain poorly understood. Here, we test the hypothesis that species of nectar-inhabiting microbes differentially influence pollinator attraction and feeding via microbial metabolites produced in nectar. We first examined the electrophysiological potential for bumblebee (*Bombus impatiens*) antennal olfactory neurons to respond to microbial volatile organic compounds (mVOCs), followed by an olfactory preference test. We also assessed gustatory preferences for microbial-altered nectar through both no-choice and choice feeding assays. Antennal olfactory neurons responded to some mVOCs, and bees preferred nectar solutions inoculated with the bacterium *Asaia astilbes* over the yeast *Metschnikowia reukaufii* based on volatiles alone. However, *B. impatiens* foragers consumed significantly more *Metschnikowia*-inoculated nectar, suggesting distinct roles for mVOCs and non-volatile metabolites in mediating both attraction and feeding decisions. Collectively, our results suggest that microbial metabolites have significant potential to shape interspecific, plant–pollinator signalling, with consequences for forager learning, economics and floral host reproduction.

1. Introduction

To successfully persist in a chemosensory environment, animals must receive and interpret cues and signals of ecologically important information, such as the quantity and quality of resources potentially available to them [1]. This is especially true of pollinators such as bumblebees, which integrate multi-modal signals, including form, colour and scent, to accurately identify rewarding flowers [2]. Like other food resources, flowers host varied microbial species and communities [3,4], which produce metabolites that may act as cues of resource availability and quality, with consequences for pollinator foraging [5,6]. Indeed, insect pollinators are highly sensitive to shifts in volatile organic compound abundance and identity [7–9], with scents known to both influence foraging preferences and mediate learning [10]. However, the role of microbial volatile organic compounds (mVOCs), as well as those that are non-volatile, in mediating pollinator attraction and foraging decisions still remains largely unclear.

In standing crop nectar, bacteria and fungi colonize between 20 and 70% of individual flowers and can reach densities exceeding 10^7 and 10^5 cells μl^{-1}, respectively [3,4]. Upon colonization, these microbes metabolize sugars and

amino acids [5,11], affecting pollinator foraging and plant reproduction [5,12,13]. Intense competition between microbes in nectar often results in flowers that are dominated by either yeast or bacteria [14]. Yeasts and bacteria differ in mVOC composition and acceptance to pollinators [6], but also differentially influence non-volatile nectar traits [15] and shift pollinator perceptions of nectar quality [16]. Predicting microbial effects on pollinator foraging and behaviour requires examining responses to olfactory (headspace mVOCs) and gustatory (dissolved chemicals) cues.

Here, we test the hypothesis that yeasts and bacteria differentially influence bumblebee attraction and feeding. Bumblebees (*Bombus impatiens*) represent an ideal animal system for testing this hypothesis, owing to their close ecological and evolutionary relationships with yeasts [17,18] and bacteria [19,20]. In this study, we addressed the following research questions. First, can bumblebees perceive mVOCs? And if so, how do they affect preference? Second, how do nectar-inhabiting microbes influence bumblebee gustation? Finally, how does gustation experience influence bumblebee preferences for mVOCs? Through the use of electroantennography (EAG), olfactometer (Y-tube) bioassays, and choice and no-choice gustation assays, we discovered that bumblebees exhibit distinct preferences for mVOCs versus gustatory cues, with microbial metabolites informing foraging decisions in both a species-specific manner and modality.

2. Material and methods

(a) Study system

We used three colonies of the generalist bumblebee *Bombus impatiens* (Koppert Biological Systems, Inc.; Howell, MI, USA) and a single strain each of the nectar-inhabiting yeast *Metschnikowia reukaufii* (Metschnikowiaceae; GenBank ID: MF319536) and bacterium *Asaia astilbes* (Acetobacteraceae; GenBank ID: KC677740). Both *Metschnikowia* and *Asaia* are commonly isolated from floral nectar [21] and pollinators [22], but are known to differentially influence nectar chemistry and scent [23]. With respect to nectar chemistry, prior work has revealed that *Asaia* can cause greater reductions in nectar pH than *Metschnikowia*, while also simultaneously increasing glucose and fructose concentrations to a greater degree [22]. As for scent, the mVOC blend emitted by *Metschnikowia* is characterized by esters, including ethyl butyrate, 2-methylpropyl acetate and 3-methylbutyl acetate, and the alcohols 2-butanol and 3-ethoxy-1-propanol, and a relatively greater abundance of ethyl acetate, and alcohols 3-methyl-1-butanol, 2-methyl-1-butanol, 3-methyl-3-buten-1-ol, ethanol and 2-phenylethanol. *Asaia* emits many of these same compounds, albeit at significantly lower concentrations. The *Asaia* mVOC profile is also distinguished by the presence of the metabolite 2,5-dimethylfuran [6].

(b) Experiment 1: can bumblebees perceive microbial volatile organic compounds?

We examined responses of antennal olfactory neurons to mVOCs produced by *Metschnikowia* and *Asaia* (table 1) by puffing each metabolite (0.4 µmol) over excised *B. impatiens* antennae ($N = 6$/metabolite), following an established protocol [6]. Recorded antennal responses were standardized using responses to both blanks and a positive control stimulus (0.4 µmol geraniol). For additional details, see electronic supplementary material, S1 Methods. To assess which mVOCs were detected by bumblebees, we used *t*-tests with false discovery rate correction to examine if normalized EAG responses were significantly different from zero (i.e. no detectable response). All analyses here and below were performed in R (v. 3.5.2) [24].

(c) Experiment 2: how do microbial volatile organic compounds influence bumblebee preference?

To assess whether bumblebees exhibit an innate preference when exposed to mVOCs, we used an olfactometer assay (Y-tube; electronic supplementary material, figure S1), with the assay performed under red light. Naive bumblebees housed at the University of California, Davis were starved for 6 h, then released individually into the Y-tube. For each bee, both initial choice and time spent in each arm were recorded, with the assay repeated twice for each bee. Across assays, the treatment assignment for each arm was reversed, with preference measured over a 5 min period. Treatments consisted of synthetic nectar (3% w/v sucrose; 6% w/v each of glucose and fructose; 0.1 mM each of glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline and L-serine) [25,26], inoculated with each respective microbe at an initial density of 10^3 cells μl^{-1} from actively growing subcultures, and incubated at 29°C for 4 days. This initial density is a magnitude or more below averages typically observed in flowers in the field [3,4]. Bees tested were both fed and treated similarly to those used for EAG assays. Overall, a total of 32 bees were tested, and sourced from two colonies. For additional experimental details, see electronic supplementary material, S1 Methods. To determine if bees have a preference for different microbes, data were analysed with a binomial mixed-model for first choice, implemented with the *lme4* package [27]. Bee identity and source colony were treated as random effects. A linear mixed-effect (LME) model was used for time spent in each arm, implemented with the *nlme* package [28], with microbial treatment as a fixed effect, and bee individual and colony source as random effects.

(d) Experiment 3: how do nectar-inhabiting microbes influence bumblebee gustation?

To first assess gustatory preferences of bumblebees ($N = 42$ bees from two colonies) for nectar colonized by microbial taxa, we used a no-choice feeding assay. In this assay, bees were housed in individual vials with modified lids that accommodated a feeding apparatus (electronic supplementary material, figure S2). The feeding apparatus consisted of attached vials, filled with 1 ml of either *Asaia*- or *Metschnikowia*-treated nectar. Before presentation to foragers, these vials were weighed, and then bees were allowed to feed for 24 h, after which tubes were re-weighed to determine consumption. Additional vials without bees were included to account for potential differences in evaporation among nectar treatments ($N = 3$ per treatment). For details, see electronic supplementary material, S1 Methods. We used a *t*-test to assess how nectar consumption was affected by the nectar treatment.

(e) Experiment 4: how does gustation experience influence bumblebee preferences for microbial volatile organic compounds?

Because bees exhibited marked differences in preference for mVOCs versus gustatory cues (see Results below), we also assessed how exposure to gustatory cues influenced bee preference for mVOCs ($N = 24$ bees from two colonies). Individual foragers were subjected to the olfactometer assay (electronic supplementary material, figure S1), then a gustatory choice assay where individual bees were housed in a feeding chamber, consisting of approximately 9 cm of perforated tubing, with feeding vials on either

Table 1. Volatile organic compounds produced by nectar-inhabiting microorganisms and their respective normalized mean bumblebee electroantennogram (EAG) response ± s.e. ($N = 6$) and corresponding false discovery rate corrected *p*-values.

		peak area in microbial headspace[a] ($\times 10^5$)		normalized EAG response[b]	
class	chemical	*A. astilbes*	*M. reukaufii*	(%; $N = 6$ bees)	*p*-value
1° alcohol	ethanol	23 ± 8	6800 ± 200	−12 ± 14	0.72
	n-propanol	0	30 ± 2	−2 ± 4	0.80
	2-methylpropanol	1.5 ± 0.8	614 ± 3	−8 ± 6	0.67
	2-methyl-1-butanol	44 ± 2[c]	6990 ± 80[c]	−12 ± 14	0.72
	3-methyl-1-butanol			−7 ± 9	0.72
	3-methyl-3-buten-1-ol	0.88[d]	5.5 ± 0.2	−9 ± 9	0.72
	4-penten-1-ol	0	8.9 ± 0.6	−5 ± 5	0.72
	n-hexanol	5.1 ± 0.3	6 ± 2	66 ± 42*	0.047
	3-ethoxy-1-propanol	0	1.8 ± 0.4	−4 ± 14	0.80
	2-ethyl-1-hexanol	77 ± 6	29 ± 2	144 ± 8***	0.00025
	2-phenylethanol	4.7 ± 0.5	260 ± 20	73 ± 13*	0.022
2° alcohol	2-butanol	0	10 ± 1	−5 ± 8	0.72
aldehyde	acetaldehyde	3 ± 2	96 ± 7	23 ± 7†	0.07
ester	ethyl acetate	0	130 ± 10	−5 ± 7	0.72
	2-methylpropyl acetate	0	5.3 ± 0.6	20 ± 7	0.11
	ethyl butyrate	0	6 ± 1	−10 ± 18	0.76
	3-methylbutyl acetate	0	41 ± 2	24 ± 6*	0.047
isoprenoid	isoprene	9 ± 1	0	−1 ± 9	0.93
ketone	3-hydroxy-2-butanone	15 ± 1	53 ± 0.9	54 ± 35	0.52
misc	2,5-dimethylfuran	16 ± 4	0	−5 ± 15	0.80

[a]Relative abundance of volatiles in microbial headspace after 96 h growth in synthetic nectar as reported in [6], excluding unknown or unconfirmed compounds.
[b]Normalized mean response is significantly different from 0 (false discovery rate †$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
[c]Peak areas for the isomers 2-methyl-1-butanol and 3-methyl-1-butanol are summed as a result of co-elution and common fragmentation patterns.
[d]Compound observed in only one replicate on day 4.

end of the chamber (electronic supplementary material, figure S3) for 24 h, each containing a different microbial-conditioned nectar. Vials were weighed to determine nectar consumption. Bees were then subjected to a second olfactometer assay. In order to determine whether olfactory preferences changed before and after gustation experience, we fit an LME model with proportion of time spent in olfactometer arms as the response variable, nectar treatment and choice test order as fixed effects, and bee individual and source colony as random effects. An interaction between choice test order and nectar treatment was also included as a term in the model. Bumblebee feeding preferences were also analysed with an LME model, with amount consumed as the response variable, nectar treatment as a fixed effect, and bee individual and source colony as random effects.

3. Results

(a) Experiment 1: can bumblebees perceive microbial volatile organic compounds?

Bumblebee olfactory neurons were highly sensitive to a subset (4/20) of mVOCs tested through EAG (table 1), including 1-hexanol, 2-ethyl-1-hexanol, 2-phenylethanol and 3-methylbutyl acetate. Notably, the alcohol 2-ethyl-1-hexanol elicited the strongest EAG depolarization response, surpassing that of the positive control (geraniol at 0.4 µmol).

(b) Experiment 2: how do microbial volatile organic compounds influence bumblebee preference?

Naive bees spent on average approximately 67% of their time in Y-tube arms assigned to *Asaia* (figure 1*a*; $F_{1,64} = 21.52$, $p < 0.0001$). Despite this clear preference, no detectable signal was observed for first choice ($p = 0.67$).

(c) Experiment 3: how do nectar-inhabiting microbes influence bumblebee gustation?

In this no-choice assay, after accounting for evaporation, bees consumed approximately 50% more *Metschnikowia*-conditioned nectar (figure 1*b*; $t_{29.5} = -2.70$, $p = 0.011$).

(d) Experiment 4: how does gustation experience influence bumblebee preferences for microbial volatile organic compounds?

Bumblebees spent approximately 15% more of their time in the Y-tube arm assigned to *Asaia* in the first olfactometer test ($F_{1,163} = 9.09$, $p = 0.003$). These same bees consumed

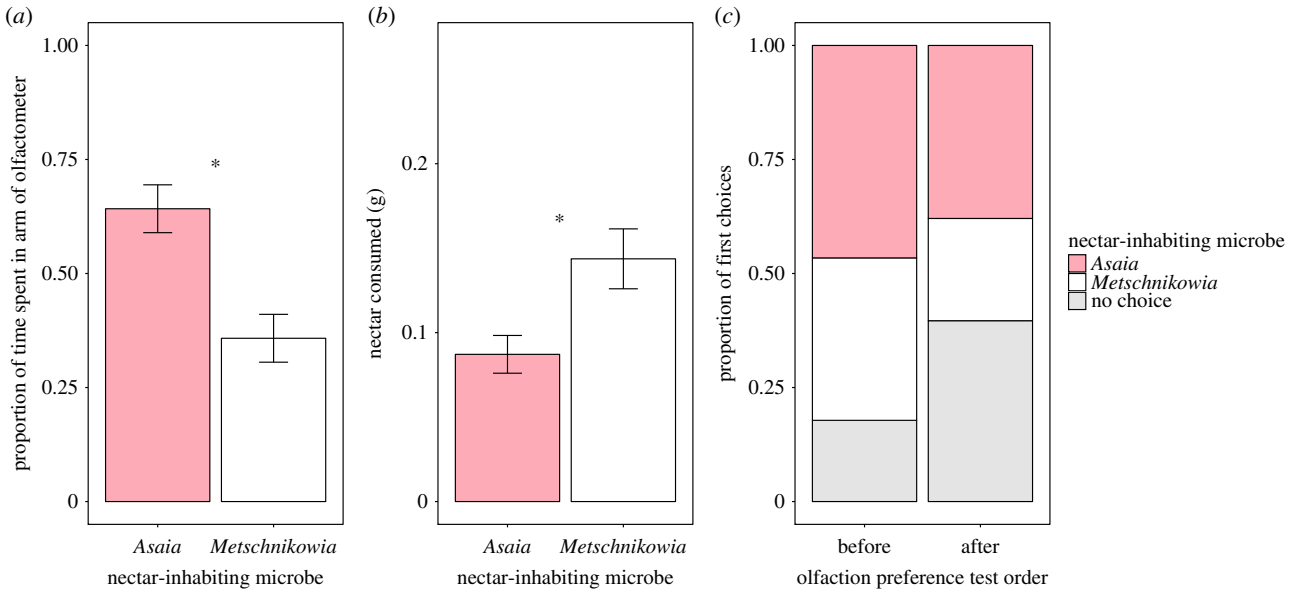

Figure 1. Behavioural (*a*; Experiment 2), gustatory (*b*; Experiment 3) and gustation experience-informed (*c*; Experiment 4) preferences of bumblebees for artificial nectar colonized by nectar-inhabiting microbes (pink, *Asaia astilbes*; white, *Metschnikowia reukaufii*) and the volatile and non-volatile organic compounds they emit.

approximately 50% more *Metschnikowia*-conditioned nectar when presented with a choice ($F_{1,46} = 12.29$, $p = 0.001$), mirroring results observed in Experiment 3. After experiencing these microbial olfactory and gustatory cues however, bees reduced the frequency (albeit non-significant) with which they chose the *Asaia* mVOC blend in the second olfactometer test (figure 1*c*; $\chi^2 = 0.83$, $p = 0.41$), with many foragers making 'no choice' at all (i.e. remained active in the main channel of the olfactometer). These bees also increased the amount of time spent in the *Metschnikowia* arm of the olfactometer, but this increase was not significant ($F_{1,163} = 0.11$, $p = 0.74$).

4. Discussion

Collectively, our results indicate that volatile and non-volatile microbial metabolites can shape interspecific, plant–pollinator signalling. More specifically, microbial VOCs were both perceived by and could influence bee preference. Across behavioural assays, bees were more attracted to the mVOC blend produced by the bacterium *Asaia* over yeast *Metschnikowia*. We hypothesize that the metabolite 2-ethyl-1-hexanol, which elicited the strongest EAG depolarization response, may play a role in mediating this response: *Asaia* emits nearly twice the amount of this mVOC [6]. The gustatory preference exhibited by bees, however, was distinct from that observed in olfactory tests. Across all feeding assays performed, bumblebees consistently consumed more *Metschnikowia*-conditioned nectar. We suspect that this aversion to the taste of *Asaia*-conditioned nectar may be driven by metabolites dissolved in nectar, such as acetic acid. *Asaia* is known to significantly reduce nectar pH [22,23], likely through production of this organic acid. Though volatile, acetic acid was not detected in our previous screening of *Asaia* mVOCs [6] and we believe that due to its high aqueous solubility, it remained primarily dissolved in nectar tested and presented to foragers. Finally, our results suggest that bees may integrate experiences of volatile and non-volatile metabolites to inform future foraging decisions; however, future experimentation is required that explicitly disentangles exposure to these metabolites and how they collectively influence associative learning in bee pollinators.

In natural systems, bees must navigate a chemosensory landscape partly shaped by microbial associates of floral hosts. Though bumblebees may display innate preferences for particular mVOCs, as suggested in our naive forager olfaction test, foragers have potential to develop learned preferences for microbial metabolites through repeated exposure to both the scent and taste of yeast or bacterial-colonized nectar. Such preferences may manifest to affect patterns of floral constancy and the quantity and quality of benefits exchanged in these mutualistic interactions. It remains to be determined however whether pollinators benefit from microbial-derived cues, such as improved foraging efficiency through localization of resources. Alternatively, these cues may be more exploitative, and benefit microbes that rely upon pollinator dispersal to reach new floral habitats [29]. Such outcomes may hinge on both the identity and density of the microbial species encountered, where varied immigration histories can give rise to divergent microbial communities both within flowers of a host and among other species. Our results demonstrate that future investigations on the evolutionary ecology of floral signalling should consider the multiple ways in which microbes influence host phenotype and the innate and learned response of pollinators.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c4877tc [30].

Authors' contributions. R.N.S., C.C.R., J.J.B. and R.L.V. conceived the study. R.N.S., C.C.R. and I.M. collected data, while R.N.S. and C.C.R. performed statistical analyses and drafted the manuscript. All authors contributed to manuscript editing, gave final approval for publication and agree to be held accountable for the worked performed therein.

Competing interests. We declare we have no competing interests.

Funding. This research was supported by UC Davis and Hatch NE1501 awarded to R.V., USDA-ARS Research Project 6036-22000-028 (J.B. and C.R.), and 2016 ARS Administrator Research Associate programme (C.R.). R.S. acknowledges support from a USDA NIFA Education and Literacy Initiative Postdoctoral Fellowship (2017-67012-26104).

Acknowledgements. We thank M. Handy, A. Khan, I. Munkres, H. Pathak and M. Anderson for laboratory assistance, and B. Forguson for greenhouse assistance. We also thank three anonymous reviewers for constructive comments on a prior version of this manuscript.

References

1. Carthey AJR, Gillings MR, Blumstein DT. 2018 The extended genotype: microbially mediated olfactory communication. *Trends Ecol. Evol.* **33**, 885–894. (doi:10.1016/j.tree.2018.08.010)
2. Schiestl FP, Johnson SD. 2013 Pollinator-mediated evolution of floral signals. *Trends Ecol. Evol.* **28**, 307–315. (doi:10.1016/j.tree.2013.01.019)
3. Herrera CM, de Vega C, Canto A, Pozo MI. 2009 Yeasts in floral nectar: a quantitative survey. *Ann. Bot.* **103**, 1415–1423. (doi:10.1093/aob/mcp026)
4. Fridman S, Izhaki I, Gerchman Y, Halpern M. 2012 Bacterial communities in floral nectar. *Environ. Microbiol. Rep.* **4**, 97–104. (doi:10.1111/j.1758-2229.2011.00309.x)
5. Vannette RL, Gauthier MPL, Fukami T. 2013 Nectar bacteria, but not yeast, weaken a plant–pollinator mutualism. *Proc. R. Soc. B* **280**, 20122601. (doi:10.1098/rspb.2012.2601)
6. Rering CC, Beck JJ, Hall GW, McCartney MM, Vannette RL. 2018 Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytol.* **220**, 750–759. (doi:10.1111/nph.14809)
7. Dobson HEM. 2006 Relationship between floral fragrance composition and type of pollinator. In *Biology of floral scent* (eds N Dudareva, E Pichersky), pp. 147–198. Boca Raton, FL: CRC Press.
8. Pichersky E, Gershenzon J. 2002 The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr. Opin Plant Biol.* **5**, 237–243. (doi:10.1016/S1369-5266(02)00251-0)
9. Galen C, Kaczorowski R, Todd SL, Geib J, Raguso RA. 2011 Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the alpine skypilot *Polemonium viscosum*. *Am. Nat.* **177**, 258–272. (doi:10.1086/657993)
10. Wright GA, Schiestl FP. 2009 The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Funct. Ecol.* **23**, 841–851. (doi:10.1111/j.1365-2435.2009.01627.x)
11. Schaeffer RN, Vannette RL, Irwin RE. 2015 Nectar yeasts in *Delphinium nuttallianum* (Ranunculaceae) and their effects on nectar quality. *Fungal Ecol.* **18**, 100–106. (doi:10.1016/j.funeco.2015.09.010)
12. Herrera CM, Pozo MI, Medrano M. 2013 Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. *Ecology* **94**, 273–279. (doi:10.1890/12-0595.1)
13. Schaeffer RN, Irwin RE. 2014 Yeasts in nectar enhance male fitness in a montane perennial herb. *Ecology* **95**, 1792–1798. (doi:10.1890/13-1740.1)
14. Toju H, Vannette RL, Gauthier M-PL, Dhami MK, Fukami T. 2018 Priority effects can persist across floral generations in nectar microbial metacommunities. *Oikos* **127**, 345–352. (doi:10.1111/oik.04243)
15. Vannette RL, Fukami T. 2018 Contrasting effects of yeasts and bacteria on floral nectar traits. *Ann. Bot.* **121**, 1343–1349. (doi:10.1093/aob/mcy032)
16. Vannette RL, Fukami T. 2016 Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. *Ecology* **97**, 1410–1419. (doi:10.1890/15-0858.1)
17. Schaeffer RN, Mei YZ, Andicoechea J, Manson JS, Irwin RE. 2017 Consequences of a nectar yeast for pollinator preference and performance. *Funct. Ecol.* **31**, 613–621. (doi:10.1111/1365-2435.12762)
18. Pozo MI, Bartlewicz J, van Oystaeyen A, Benavente A, van Kemenade G, Wäckers F, Jacquemyn H. 2018 Surviving in the absence of flowers: do nectar yeasts rely on overwintering bumblebee queens to complete their annual life cycle? *FEMS Microbiol. Ecol.* **94**, fiy196. (doi:10.1093/femsec/fiy196)
19. Koch H, Abrol DP, Li J, Schmid-Hempel P. 2013 Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Mol. Ecol.* **22**, 2028–2044. (doi:10.1111/mec.12209)
20. Graystock P, Rehan SM, McFrederick QS. 2017 Hunting for healthy microbiomes: determining the core microbiomes of *Ceratina*, *Megalopta*, and *Apis* bees and how they associate with microbes in bee collected pollen. *Conserv. Genet.* **18**, 701–711. (doi:10.1007/s10592-017-0937-7)
21. Lachance M-A, Starmer WT, Rosa CA, Bowles JM, Barker JSF, Janzen DH. 2001 Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Res.* **1**, 1–8. (doi:10.1111/j.1567-1364.2001.tb00007.x)
22. Good AP, Gauthier M-PL, Vannette RL, Fukami T. 2014 Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. *PLoS ONE* **9**, e86494. (doi:10.1371/journal.pone.0086494)
23. Lenaerts M *et al.* 2017 Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. *Funct. Ecol.* **31**, 2061–2069. (doi:10.1111/1365.2435.12933)
24. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/.
25. Baker HG, Baker I. 1982 Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In *Biochemical aspects of evolutionary biology. Proc. 4th Ann. Spring syst. Symp.* (ed. MH Nitecki), pp. 131–171. Chicago, IL: University of Chicago Press.
26. Gardener MC, Gillman MP. 2001 Analyzing variability in nectar amino acids: composition is less variable than concentration. *J. Chem. Ecol.* **27**, 2545–2558. (doi:10.1023/A:1013687701120)
27. Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)
28. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2018 *nlme: Linear and nonlinear mixed effects models*. See https://CRAN.R-project.org/package=nlme.
29. Madden AA, Epps MJ, Fukami T, Irwin RE, Sheppard J, Sorger DM, Dunn RR. 2018 The ecology of insect–yeast relationships and its relevance to human industry. *Proc. R. Soc. B* **285**, 20172733. (doi:10.1098/rspb.2017.2733)
30. Schaeffer RN, Rering CC, Maalouf I, Beck JJ, Vannette RL. 2019 Data from: Microbial metabolites elicit distinct olfactory and gustatory preferences in bumblebees. Dryad Digital Repository. (https://doi.org/10.5061/dryad.c4877tc)