

NOTE

# Individual Dietary Specialization in a Generalist Bee Varies across Populations but Has No Effect on the Richness of Associated Microbial Communities

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**ABSTRACT:** Despite the increasingly documented occurrence of individual specialization, the relationship between individual consumer interactions and diet-related microbial communities in wild populations is still unclear. Using data from nests of *Ceratina australensis* from three different wild bee populations, we combine metabarcoding and network approaches to explore the existence of individual variation in resource use within and across populations and whether dietary specialization affects the richness of pollen-associated microbes. We reveal the existence of marked dietary specialization. In the most specialized population, we also show that individuals' diet breadth was positively related to the richness of fungi but not bacteria. Overall, individual specialization appeared to have a weak or negligible effect on the microbial richness of nests, suggesting that different mechanisms beyond environmental transmission may be at play regarding microbial acquisition in wild bees.

**Keywords:** small carpenter bee, diet breadth, native bee, network centrality, pollen metabarcoding, plant-pollination network.

## Introduction

Community ecology has traditionally studied species' niches under the assumption that generalist species are composed of generalist populations, which, in turn, are composed of generalist individuals. The underlying mechanism behind this assumption is that individuals within a species are ecologically equivalent. However, the occurrence of individual

specialization has been increasingly documented, illustrating that populations are, in fact, composed of ecologically heterogeneous individuals (Bolnick et al. 2010, 2011; Araújo et al. 2011; Ingram et al. 2018; Cecala and Wilson Rankin 2020). Although studies of individual variation in resource use have focused primarily on vertebrate species (Bolnick et al. 2003), such variation has also been documented in several invertebrate species, including social bees (Heinrich 1976; Dupont et al. 2011, 2014; Tur et al. 2014).

Individual-level specialization may affect consumers beyond resource acquisition, as different resource species harbor different nonresource species. For example, diet breadth can affect exposure and acquisition of microbes, both beneficial and pathogenic, which can affect several life-history aspects (Egerton et al. 2018; Kartzinel et al. 2019). Dietary breadth can, therefore, affect the composition and function of the gut microbiome across diverse hosts, including vertebrates and insects (Bolnick et al. 2014; Sanders et al. 2017; Douglas 2018; Egerton et al. 2018; Youngblut et al. 2019). At the species level, wider diet breadth in bees has been linked to lower pathogen prevalence (Figueroa et al. 2020), but how individual diet breadth affects the acquisition of fungal and bacterial associates remains unstudied. The link between consumer interactions of individuals and microbial transmission is, therefore, still unclear, especially in wild communities (Daszak et al. 2000). Given that managed and wild bees are essential pollinators (Klein et al. 2007; Garibaldi et al. 2013), understanding the patterns behind microbial transmission across populations would help ensure the maintenance of essential ecosystem services.

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Flowers harbor a diversity of microbes (Herrera et al. 2008; McArt et al. 2014; Vannette 2020), and by foraging on different flowers, pollinators act as vectors, transporting microbes from flower to flower (McArt et al. 2014). For example, flowers commonly visited by pollinators harbor more microbes than unvisited flowers (Aizenberg-Gershtein et al. 2013), and floral microbes emit volatiles that affect pollinator visitation (Rering et al. 2018). Shared microbes between flowers and wild bee nests show signatures of selection on genes that could be beneficial to the host (Vuong and McFrederick 2019), and pollen-borne microbes appear to be essential for the development of some bees (Dharampal et al. 2019). Thus, by visiting flowers, bees may be acting as microbial transmission vectors (Graystock et al. 2015; McFrederick et al. 2017), suggesting the existence of a link between dietary breadth, plant-bee interactions, and microbial transmission.

Foraging patterns in wild bee populations are mainly studied through active sampling of interactions or by analyzing pollen samples from collected specimens (Otterstatter and Thomson 2007; Dupont et al. 2011, 2014; Tur et al. 2014), resulting in data from the specimens' last interaction or last foraging bout, respectively. A comparatively underemployed approach is to use the pollen provisions within bee nests (McFrederick and Rehan 2016) to understand plant-pollinator interactions. Nests harbor resources collected over several foraging bouts over multiple days, enabling us to explore individual variation in species' diet breadths through repeated samples from the same individuals, providing a holistic estimate of an individual's foraging history. The alfalfa leafcutter bee, for example, may visit 2,550 flowers to provision a single brood cell (Klostermeyer and Gerber 1969). While priority effects and filtering will likely affect bee-nest microbial community composition, foraging patterns may have strong effects on the microbes that are inoculated into the nests in the first place. Thus, analyzing the pollen samples of individual nests provides an ideal opportunity to test hypotheses about individual variation in foraging patterns. Because nests are difficult to locate (Sardiñas and Kremen 2014), studies using nest pollen samples to investigate variation in foraging patterns of bee individuals are scarce.

The genus *Ceratina* comprises small carpenter bees present on all continents except Antarctica and is composed of more than 200 species that collect pollen and nectar from various plant species (Dew et al. 2020). Each individual female builds a nest composed of separate brood cells in which the female provisions a pollen ball resulting from several independent foraging bouts before laying a single egg (Rehan et al. 2010). Using a metabarcoding approach, we characterized the diet composition at the individual and population levels of *Ceratina australensis* from three different populations, along with the

fungal and bacterial composition of the nest-associated pollen. In previous work, McFrederick and Rehan (2019) showed that pollen, fungal, and bacterial communities varied across habitat types and that plant communities were correlated with microbial communities, especially fungi. Here, we tested three hypotheses about the relationship between individual variation and nest microbial composition. First, we explored whether (H1) *Ceratina* individuals differ in their resource use at the individual level, within and across populations; the existence of intraspecific variation would indicate that generalist individuals have wider niche breadths, potentially increasing microbial exposure. We then tested the prediction that (H2) pollen from nests of more generalized individuals would present more species-rich microbial communities than more specialized individuals. Finally, we addressed whether (H3) dietary breadth and the richness of microbial communities are associated.

## Methods

### *Study Sites and Data Collection*

We reanalyzed data previously published by McFrederick and Rehan (2019) from nests of *Ceratina australensis* collected in January 2015. We analyzed 87 pollen provisions from 38 nests from *C. australensis* from three different populations in Australia (Oppenheimer and Rehan 2021): 18 from South Australia, 11 from Victoria, and nine from Queensland, representing all the nests for which pollen barcode data were reliably obtained (McFrederick and Rehan 2019). Each population was sampled for 1 week, and all nests were in the active brood stage, with females actively collecting pollen for pollen balls and laying eggs. *Ceratina australensis* populations are composed of predominantly solitary individuals (Rehan et al. 2010, 2011), and on the rare occasion that two females are present in the nest, only one is responsible for reproduction and foraging (Rehan et al. 2014).

We extracted DNA from each pollen provision using protocols previously described by McFrederick and Rehan (2019; for details, see the supplemental PDF). We considered only sequences with high similarity (>80% confidence using the scikit-learn or RDP classifiers) to the closest reference sequence. For each sample (i.e., pollen provision in a nest), we removed sequences that represented less than 1% of averaged reads, a commonly used threshold (Bison et al. 2015; Pansu et al. 2019) under which sequences are considered as sequencing error or an occasional resource. We pooled all provisions within a nest as an independent measure of an individual's resource use. We then randomly subsampled to the same number of reads across all samples, with different rarefaction depths for each taxa (McFrederick and Rehan 2019;

supplemental PDF). Because of the quality filtering and rarefying, data availability differs across individuals, resulting in a different number of data points for plants, bacteria, and fungi (tables S1, S2).

#### Calculating Individual Variation

To explore the existence of individual specialization within and across populations (H1), we used the rarefied plant data at the genus level to calculate the proportional similarity (PS) index (Schoener 1968; Roughgarden 1979; Zaccarelli et al. 2013) of each  $i$  individual (supplemental PDF). To test whether binning plants at the generic level affected individual specialization, we also performed all analyses using amplicon sequence variants (ASVs), and the results remain unchanged (supplemental PDF). The  $PS_i$  index ranges from 0 to 1, where the smaller the value, the greater individual specialization, indicating individuals that consume an item that no one else in the population consumes (Bolnick et al. 2002). We used the R package RInSp (Zaccarelli et al. 2013) to calculate  $PS_i$ .

To evaluate whether individual variation in diet differs from the null expectation that each individual randomly chooses their resources (Bolnick et al. 2002), for each individual at each population, we randomly assigned interactions with plants to different bee individuals using a Monte Carlo sampling approach and recalculated  $PS_i$  values. We created 9,999 replicates for each population and computed  $P$  values by calculating how many times the empirical  $PS_i$  values were observed in the null communities.

#### Calculating Microbial Richness

From the rarefied nest-level data (see above), we calculated the richness of fungi and bacteria of each individual nest as the number of unique ASVs present. It is worth pointing out that there were no differences in beta diversity on the fungi and bacterial communities associated with brood cells within each nest (McFrederick and Rehan 2019), suggesting that bacterial succession was not a main driver of community structure within a nest.

#### Calculating Resource Overlap among Individuals

To test whether dietary breadth and the richness of microbial communities are associated (H3), we used a network approach to characterize individuals' resource overlap by calculating weighted-closeness centrality (Blüthgen et al. 2006; Cirtwill et al. 2018). Weighted-closeness centrality (hereafter, centrality) measures the sum of the length of shortest paths between a focal individual and every other individual in the network. The larger an individual's cen-

trality, the closer they are to all other individuals in the network and the more they interact with plants that other individuals also interact with, thus increasing the potential of microbial transmission. We used the bipartite package (Dormann et al. 2008) to calculate centrality.

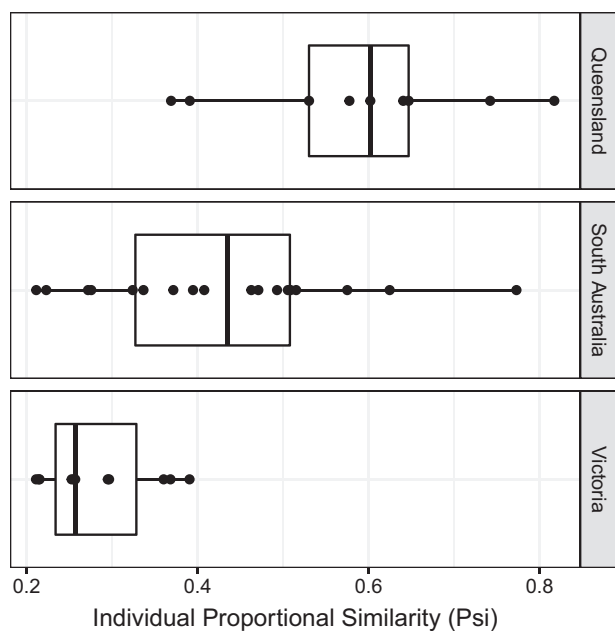
#### Statistical Analyses

To analyze whether  $PS_i$  values differed among populations (H1), we used analysis of variance (ANOVA) for which all assumptions were met. We used sequential ANOVA (type I) and linear models to quantify the effects of individual specialization (H2) and centrality (H3) on microbial richness. Analyses on log-transformed microbial richness were qualitatively the same (figs. S9, S11). We conducted separate analyses for pollen-associated bacteria and fungi. To quantify the effects of bee specialization (H2), we included sample site as a covariate and tested for both main and site-dependent effects of specialization. To quantify the effects of centrality, we included sample site and degree (number of plants each bee individual interacted with) and site-by-degree interaction as covariates to isolate the effect of centrality on microbial richness. We also tested for site-dependent effects of centrality. Here, we report results from microbial richness rarefied to read depths used by McFrederick and Rehan (2019), but we also point out whether results were (in)consistent across different levels of rarefaction (lower read depth that enables us to include 90% of the data without rarefying the data; supplemental PDF). We performed all analyses in R (ver. 4.0.0; R Core Team 2020).

### Results

Bee individuals exhibited clear differences in resource use both within ( $PS_i$  null model: all  $P$  values  $< .001$ ; figs. 1, S4–S6), and across populations (ANOVA:  $F_{2,35} = 14.09$ ,  $P < .001$ ; figs. 1, S5; H1). The Victoria population was the most specialized, followed by South Australia and Queensland (figs. 1, S4–S6). These results were robust despite the small sample sizes (figs. S2, S3).

We expected a negative effect of specialization on microbial richness (H2), but we found little evidence for this hypothesis regardless of the read depth we rarefied our richness estimates to (fig. S8; tables S1, S2). For example, after accounting for site-level differences in bacterial richness ( $F_{2,28} = 5.64$ ,  $P = .009$ ), there were no clear main or site-dependent effects of specialization ( $PS_i$ :  $F_{1,28} = 1.52$ ,  $P = .227$ ; fig. S8; site  $\times$   $PS_i$ :  $F_{2,28} = 0.61$ ,  $P = .551$ ). Similarly, for fungal richness, we found clear differences among sites ( $F_{2,26} = 6.18$ ,  $P = .006$ ) but no clear main or



**Figure 1:** Dietary specialization ( $PS_i$ ) varies within populations and across populations. Each point represents  $PS_i$  values of individual nests, with brood cells combined. All empirical values were significantly smaller than expected by the null model.

site-dependent effects of specialization ( $PS_i$ ;  $F_{1,26} = 0.010$ ,  $P = .920$ ; fig. S8; site  $\times$   $PS_i$ ;  $F_{2,26} = 0.34$ ,  $P = .716$ ). While it is generally difficult to conclude that a biological factor has no effect, our estimates of uncertainty (standard error and 95% confidence interval) suggest that any effect of specialization is likely to be weak, especially for fungal richness (fig. S8).

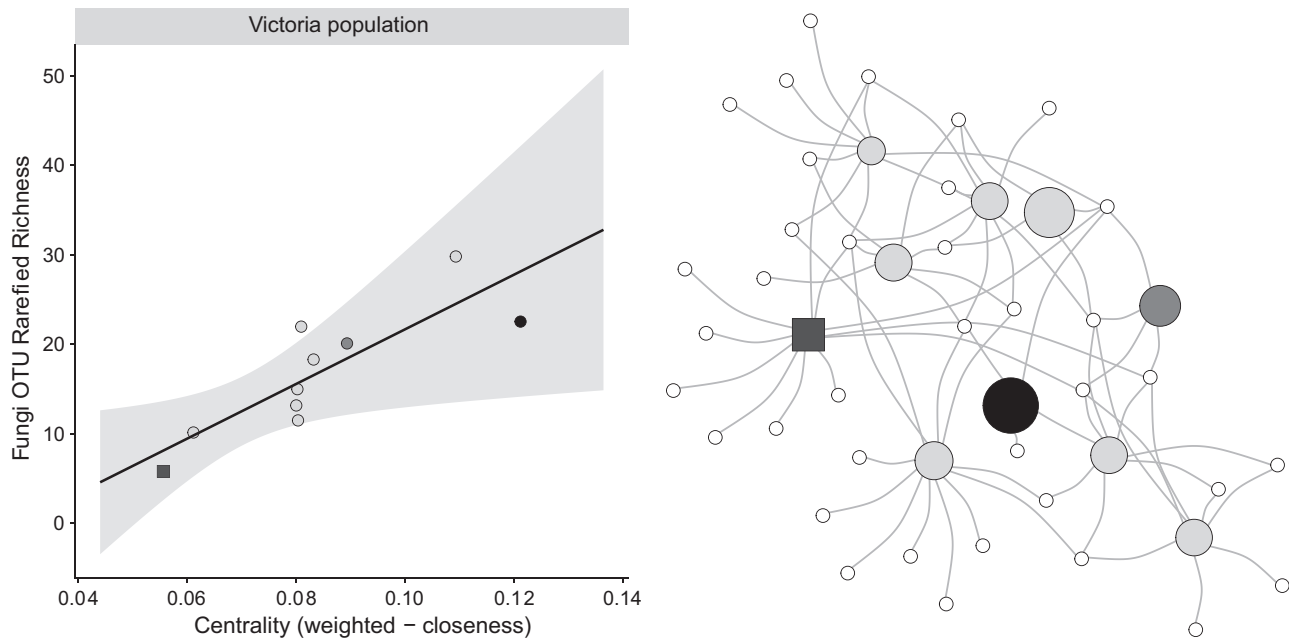
We found mixed support for the hypothesis that dietary breadth (measured as centrality) and the richness of microbial communities were associated (H3; figs. 2, S10; tables S3, S4). For example, after accounting for the effects of site and degree (and site  $\times$  degree; tables S3, S4), there was no evidence of main or site-dependent effects of centrality on bacterial richness (centrality:  $F_{1,25} = 0.91$ ,  $P = .349$ ; site  $\times$  centrality:  $F_{2,25} = 0.20$ ,  $P = .817$ ), and this result was consistent regardless of rarefaction method (fig. S10; table S3; all  $P > .210$ ). But for fungal richness, we found that the effect of bee-individual centrality varied among sites, although the clarity of this effect depended on our rarefaction method (table S4; nonrarefied:  $P = .002$ ; high read depth:  $P = .062$ ; low read depth:  $P = .011$ ). Specifically, we found consistent positive effects of centrality on fungal richness in the Victoria population (figs. 2, S10), whereas there was no consistent evidence of centrality effects on fungal richness at the other sites (fig. S10).

## Discussion

The assemblage of pollen inside a solitary bee's nest provides a chronicle of an individual bee's foraging history over the activity period of the nest. Pollen provisions are therefore a powerful tool for exploring the existence of individual dietary variation in wild populations and its associated consequences. Here, we leveraged the pollen provisioning behavior of a generalist bee species to understand the links between resource use and microbial associations. Our results are threefold. First, we revealed the existence of marked individual specialization in *Ceratina australensis* within and across populations. Second, we found that individual specialization had, at most, a biologically weak effect on fungal richness associated with pollen. Third, in the most specialized population, we found a strong, positive effect of individuals' dietary breadth on fungal richness. While our wide estimates of uncertainty are less conclusive for bacteria (figs. S8–S11), they nevertheless suggest that additional mechanisms beyond environmental transmission, such as microbial filtering (Keller et al. 2020), may be at play regarding microbial acquisition in wild populations. Our study highlights the microscopic variability of interaction networks and the existence of fluctuations of interaction patterns at a finer level (Trøjelsgaard and Olesen 2016).

Plant-pollinator interaction networks suggest that species are more flexible in their interaction partners when temporal variability is taken into account (Spiesman and Gratton 2016; CaraDonna and Waser 2020) and that this flexibility has population-level ramifications (Gaiarsa et al. 2021). At a finer scale, by looking at repeated samples of the same individuals, our results suggest that populations are composed of a combination of highly specialized and generalized individuals, with varying degrees of individual specialization even in the most specialized population (Victoria; fig. 1). This finding challenges the common practice of binning bee species into oligolectic and polylectic (i.e., pollen specialists or generalists) categories and instead suggests that more studies teasing out the importance of diet breadth at the species versus individual level are needed (Rothman et al. 2020). An exciting new research avenue is to explore whether this flexibility is intrinsic to individuals, related to intraspecific and interspecific competition, or reflects resource availability.

The mechanisms for environmental transmission of microbes for solitary bees are very different from those of social bees. In the social corbiculate bee species, microbes are mostly transmitted via direct contact between individuals within the colony (Koch and Schmid-Hempel 2011; Powell et al. 2014), which results in high host specificity (Kwong and Moran 2015). In contrast, for solitary and social species with small colonies, environmental transmission through



**Figure 2:** In the Victoria population, the centrality of bee individuals had a positive effect on the richness of fungi associated with the pollen of each nest after controlling for the degree of bee individuals (*left*,  $\beta = 305$ , standard error = 125.5,  $t_{16} = 2.43$ ,  $P = .027$ ). *Right*, plants (same size, white nodes) and bee-individual (gray nodes) interaction network of the Victoria population. Bee individuals are scaled according to their weighted closeness centrality. For visualization purposes, three bee individuals are highlighted with shading and shapes matching individuals across panels.

shared resources may represent the most important transmission mechanism (McFrederick et al. 2012, 2013; Keller et al. 2020). Our results partially support this hypothesis. Although we found no clear effect of dietary specialization on the richness of microbial communities, we found a strong, positive effect of centrality on fungal richness only in the most specialized population. Using this data set, McFrederick and Rehan (2019) found a high clustering of fungi communities across sites but a weaker clustering effect of bacterial communities. Taken together, these results suggest that resource sharing among individuals (centrality) may be an important component in the pollen-associated richness of microbial communities in more specialized populations. This pattern further suggests that resources shared by individuals may potentially influence microbial transmission in populations formed predominantly by specialized individuals, regardless of the number of flowers each individual visits. In multispecies plant-pollinator networks, pathogen prevalence was related to the number of interactions in the network (connectance) but not to species centrality (Figueroa et al. 2020). Future studies connecting individual variation to multispecies networks are necessary to better understand the interplay between microbial transmission and interaction networks.

While our study is the first to explicitly explore the role of individual-level specialization in microbial trans-

mission and acquisition, it is not the only study to examine microbes in the context of plant-pollinator networks. Voulgari-Kokota et al. (2019) used pollen and bacteria metabarcoding to create interaction networks between flower and seven megachilid bee species. The floral composition of the bees' pollen provisions significantly correlated with the bacteria found in these provisions and in larval guts, supporting the importance of floral transmission. Zemenick et al. (2021) showed that both pollinator identity and microbial species sorting in floral nectaries influenced nectar microbial community structure. Other studies that do not use an explicit network framework have also shown that floral transmission appears to be a major driver of pollen provision microbial communities (McFrederick et al. 2012, 2017; McFrederick and Rehan 2016; Rothman et al. 2019; Voulgari-Kokota et al. 2019). We further this body of work by showing the existence of individual-level specialization in populations of generalist bees. Taken together, these results highlight both the variability of a species' foraging behavior and the variability of ecological networks across space (Trojelsgaard and Olesen 2016).

Our study demonstrates that bee individuals of a widespread, common carpenter bee vary in their level of dietary specialization, both within and across populations. We also show that different mechanisms beyond environmental

transmission may be at play regarding microbial acquisition, given that an individual's level of specialization did not affect microbial richness. We note that we focused on a single bee species and did not consider floral availability, thus the links between diet and microbe acquisition may be clearer when entire communities are considered. Future work could investigate whether greater diversity in resource availability in an area leads to greater generalization in foraging behavior at the individual level. Connecting community structure to microbial transmission is crucial to understanding future trajectories of ecological communities and to guarantee ecosystem services such as pollination. It remains unclear how different fungi and bacteria affect solitary bee fitness and overall bee health. By combining metabarcoding and network approaches, our results contribute to the growing literature linking the structure of ecological communities to microbial transmission patterns.

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#### Statement of Authorship

M.P.G. conceptualized the study and organized the data. S.R. collected the data. Q.S.M. performed molecular analysis and bioinformatics. M.P.G. and M.B. analyzed the data and interpreted the results, with input from Q.S.M. and S.R. M.P.G. wrote the original draft and was responsible for review and editing. All authors contributed to revisions and gave final approval for publication.

#### Data and Code Availability.

All code used in the analyses is available on Dryad (<https://doi.org/10.5061/dryad.5dv41ns7s>; Palumbo Gaiarsa et al. 2022) and GitHub (<https://github.com/Magaiarsa/ceratinaIndDiet>). Previously published sequence data are publicly

available under NCBI/EMBL/DDBJ accession nos. SAMN08911168–SAMN08911424.

#### Literature Cited

- Aizenberg-Gershtein, Y., I. Izhaki, and M. Halpern. 2013. Do honeybees shape the bacterial community composition in floral nectar? *PLoS ONE* 8:e67556.
- Araújo, M. S., D. I. Bolnick, and C. A. Layman. 2011. The ecological causes of individual specialisation. *Ecology Letters* 14:948–958.
- Bison, M., S. Ibanez, C. Redjadj, F. Boyer, E. Coissac, C. Miquel, D. Rioux, et al. 2015. Upscaling the niche variation hypothesis from the intra- to the inter-specific level. *Oecologia* 179:835–842.
- Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. Measuring specialization in species interaction networks. *BMC Ecology* 6:1–12.
- Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* 6:183–192.
- Bolnick, D. I., T. Ingram, W. E. Stutz, L. K. Snowberg, O. L. Lau, and J. S. Paull. 2010. Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B* 277:1789–1797.
- Bolnick, D. I., L. K. Snowberg, P. E. Hirsch, C. L. Lauber, R. Knight, J. G. Caporaso, and R. Svanbäck. 2014. Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). *Ecology Letters* 17:979–987.
- Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. Darrin Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. *American Naturalist* 161:1–28.
- Bolnick, D. I., L. H. Yang, J. A. Fordyce, J. M. Davis, and R. Svanbäck. 2002. Measuring individual-level resource specialization. *Ecology* 83:2936–2941.
- CaraDonna, P. J., and N. M. Waser. 2020. Temporal flexibility in the structure of plant-pollinator interaction networks. *Oikos* 29:1369–1380.
- Cecala, J. M., and E. E. Wilson Rankin. 2020. Mark-recapture experiments reveal foraging behavior and plant fidelity of native bees in plant nurseries. *Ecology* 101:e03021.
- Cirtwill, A. R., G. V. D. Riva, M. P. Gaiarsa, M. D. Bimler, E. F. Cagua, C. Coux, and D. M. Dehling. 2018. A review of species role concepts in food webs. *Food Webs* 6:e00093.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287:443–449.
- Dew, R. M., Q. S. McFrederick, and S. M. Rehan. 2020. Diverse diets with consistent core microbiome in wild bee pollen provisions. *Insects* 11:499.
- Dharampal, P. S., C. Carlson, C. R. Currie, and S. A. Steffan. 2019. Pollen-borne microbes shape bee fitness. *Proceedings of the Royal Society B* 286:20182894.
- Dormann, C. F., B. Gruber, and J. Fründ. 2008. Introducing the bipartite package: analysing ecological networks. *R News* 8:8–11.
- Douglas, A. E. 2018. The *Drosophila* model for microbiome research. *Lab Animal* 47:157–164.
- Dupont, Y. L., K. Trøjelsgaard, M. Hagen, M. V. Henriksen, J. M. Olesen, N. M. E. Pedersen, and W. D. Kissling. 2014. Spatial structure of an individual-based plant-pollinator network. *Oikos* 123:1301–1310.

- Dupont, Y. L., K. Trøjsgaard, and J. M. Olesen. 2011. Scaling down from species to individuals: a flower-visitation network between individual honeybees and thistle plants. *Oikos* 120:170–177.
- Egerton, S., S. Culloty, J. Whooley, C. Stanton, and R. Paul Ross. 2018. The gut microbiota of marine fish. *Frontiers in Microbiology* 9:873.
- Figueroa, L. L., H. Grab, W. H. Ng, C. R. Myers, P. Graystock, Q. S. McFrederick, and S. H. McArt. 2020. Landscape simplification shapes pathogen prevalence in plant-pollinator networks. *Ecology Letters* 23:1212–1222.
- Gaiarsa, M. P., C. Kremen, and L. C. Ponisio. 2021. Pollinator interaction flexibility across scales affects patch colonization and occupancy. *Nature Ecology and Evolution* 5:787–793.
- Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339:1608–1611.
- Graystock, P., D. Goulson, and W. O. H. Hughes. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proceedings of the Royal Society B* 282:20151371.
- Heinrich, B. 1976. The foraging specializations of individual bumblebees. *Ecological Monographs* 46:105–128.
- Herrera, C. M., I. M. García, and R. Pérez. 2008. Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee–pollinated plants. *Ecology* 89:2369–2376.
- Ingram, T., R. Costa-Pereira, and M. S. Araújo. 2018. The dimensionality of individual niche variation. *Ecology* 99:536–549.
- Kartzinel, T. R., J. C. Hsing, P. M. Musili, B. R. P. Brown, and R. M. Pringle. 2019. Covariation of diet and gut microbiome in African megafauna. *Proceedings of the National Academy of Sciences of the USA* 116:23588–23593.
- Keller, A., Q. S. McFrederick, P. Dharampal, S. Steffan, B. N. Danforth, and S. D. Leonhardt. 2020. (More than) hitchhikers through the network: the shared microbiome of bees and flowers. *Current Opinion in Insect Science* 44:8–15.
- Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharnke. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B* 274:303–313.
- Klostermeyer, E. C., and H. S. Gerber. 1969. Nesting behavior of *Megachile rotundata* (Hymenoptera: Megachilidae) monitored with an event recorder. *Annals of the Entomological Society of America* 62:1321–1325.
- Koch, H., and P. Schmid-Hempel. 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the USA* 108:19288–19292.
- Kwong, W. K., and N. A. Moran. 2015. Evolution of host specialization in gut microbes: the bee gut as a model. *Gut Microbes* 6:214–220.
- McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. *Ecology Letters* 17:624–636.
- McFrederick, Q. S., J. J. Cannone, R. R. Gutell, K. Kellner, R. M. Plowes, and U. G. Mueller. 2013. Specificity between lactobacilli and hymenopteran hosts is the exception rather than the rule. *Applied and Environmental Microbiology* 79:1803–1812.
- McFrederick, Q. S., and S. M. Rehan. 2016. Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. *Molecular Ecology* 25:2302–2311.
- . 2019. Wild bee pollen usage and microbial communities co-vary across landscapes. *Microbial Ecology* 77:513–522.
- McFrederick, Q. S., J. M. Thomas, J. L. Neff, H. Q. Vuong, K. A. Russell, A. R. Hale, and U. G. Mueller. 2017. Flowers and wild megachilid bees share microbes. *Microbial Ecology* 73:188–200.
- McFrederick, Q. S., W. T. Wcislo, D. R. Taylor, H. D. Ishak, S. E. Dowd, and U. G. Mueller. 2012. Environment or kin: whence do bees obtain acidophilic bacteria? *Molecular Ecology* 21:1754–1768.
- Oppenheimer, R. L., and S. M. Rehan. 2021. Inclusive fitness of male and facultatively social female nesting behavior in the socially polymorphic bee, *Ceratina australensis* (Hymenoptera: Xylocopinae). *Annals of the Entomological Society of America* 114:627–636.
- Otterstatter, M. C., and J. D. Thomson. 2007. Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. *Oecologia* 154:411–421.
- Palumbo Gaiarsa, M., M. Barbour, and Q. McFrederick. 2022. Data from: Individual dietary specialization in a generalist bee varies across populations but has no effect on the richness of associated microbial communities. *American Naturalist*, Dryad Digital Repository, <https://doi.org/10.5061/dryad.5dv41ns7s>.
- Pansu, J., J. A. Guyton, A. B. Potter, J. L. Atkins, J. H. Daskin, B. Wursten, T. R. Kartzinel, and R. M. Pringle. 2019. Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology* 107:1355–1376.
- Powell, J. E., V. G. Martinson, K. Urban-Mead, and N. A. Moran. 2014. Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Applied and Environmental Microbiology* 80:7378–7387.
- R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rehan, S. M., M. H. Richards, M. Adams, and M. P. Schwarz. 2014. The costs and benefits of sociality in a facultatively social bee. *Animal Behaviour* 97:77–85.
- Rehan, S. M., M. H. Richards, and M. P. Schwarz. 2010. Social polymorphism in the Australian small carpenter bee, *Ceratina (Neoceratina) australensis*. *Insectes Sociaux* 57:403–412.
- Rehan, S. M., M. P. Schwarz, and M. H. Richards. 2011. Fitness consequences of ecological constraints and implications for the evolution of sociality in an incipiently social bee. *Biological Journal of the Linnean Society* 103:57–67.
- Rering, C. C., J. J. Beck, G. W. Hall, M. M. McCartney, and R. L. Vannette. 2018. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytologist* 220:750–759.
- Rothman, J. A., C. Andrikopoulos, D. Cox-Foster, and Q. S. McFrederick. 2019. Floral and foliar source affect the bee nest microbial community. *Microbial Ecology* 78:506–516.
- Rothman, J. A., D. L. Cox-Foster, C. Andrikopoulos, and Q. S. McFrederick. 2020. Diet breadth affects bacterial identity but not diversity in the pollen provisions of closely related polylectic and oligolectic bees. *Insects* 11:645.
- Roughgarden, J. 1979. *Theory of population genetics and evolutionary ecology: an introduction*. Macmillan, New York.
- Sanders, J. G., P. Lukasik, M. E. Frederickson, J. A. Russell, R. Koga, R. Knight, and N. E. Pierce. 2017. Dramatic differences in gut bacterial densities correlate with diet and habitat in rainforest ants. *Integrative and Comparative Biology* 57:705–722.
- Sardiñas, H. S., and C. Kremen. 2014. Evaluating nesting microhabitat for ground-nesting bees using emergence traps. *Basic and Applied Ecology* 5:161–168.

- Schoener, T. W. 1968. The *Anolis* lizards of Bimini: resource partitioning in a complex fauna. *Ecology* 49:704–726.
- Spiesman, B. J., and C. Gratton. 2016. Flexible foraging shapes the topology of plant-pollinator interaction networks. *Ecology* 97:1431–1441.
- Trojelsgaard, K., and J. M. Olesen. 2016. Ecological networks in motion: micro- and macroscopic variability across scales. *Functional Ecology* 30:1926–1935.
- Tur, C., B. Vigalondo, K. Trojelsgaard, J. M. Olesen, and A. Traveset. 2014. Downscaling pollen-transport networks to the level of individuals. *Journal of Animal Ecology* 83:306–317.
- Vannette, R. L. 2020. The floral microbiome: plant, pollinator, and microbial perspectives. *Annual Review of Ecology, Evolution, and Systematics* 51:363–386.
- Voulgari-Kokota, A., M. J. Ankenbrand, G. Grimmer, I. Steffan-Dewenter, and A. Keller. 2019. Linking pollen foraging of megachilid bees to their nest bacterial microbiota. *Ecology and Evolution* 9:10788–10800.
- Voulgari-Kokota, A., Q. S. McFrederick, I. Steffan-Dewenter, and A. Keller. 2019. Drivers, diversity, and functions of the solitary-bee microbiota. *Trends in Microbiology* 27:1034–1044.
- Vuong, H. Q., and Q. S. McFrederick. 2019. Comparative genomics of wild bee and flower isolated *Lactobacillus* reveals potential adaptation to the bee host. *Genome Biology and Evolution* 11:2151–2161.
- Youngblut, N. D., G. H. Reischer, W. Walters, N. Schuster, C. Walzer, G. Stalder, R. E. Ley, and A. H. Farnleitner. 2019. Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications* 10:1–15.
- Zaccarelli, N., D. I. Bolnick, and G. Mancinelli. 2013. RInSp: an R package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution* 4:1018–1023.
- Zemenick, A. T., R. L. Vanette, and J. A. Rosenheim. 2021. Linked networks reveal dual roles of insect dispersal and species sorting for bacterial communities in flowers. *Oikos* 130:697–707.

### References Cited Only in the Online Enhancements

- Abarenkov, K., R. H. Nilsson, K.-H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, et al. 2010. The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist* 186:281–285.
- Bell, K. L., V. M. Loeffler, and B. J. Brosi. 2017. An *rbcL* reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in Plant Sciences* 5:1600110.
- Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: high-resolution sample inference from illumina amplicon data. *Nature Methods* 13:581–583.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335–336.
- Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. Kulam-Syed-Mohideen, et al. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research* 37(suppl. 1):D141–D145.
- Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41:D590–D596.
- Smith, D. P., and K. G. Peay. 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLoS ONE* 9:e90234.

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“Of the many curious plants which have been given to the world by America, the pitcher plants are among the oddest.” Figured: “*Heliamphorum*.” From “How the Pitcher Plant Got Its Leaves” by Joseph F. James (*The American Naturalist*, 1885, 19:567–578).