Individual dietary specialization in a generalist bee varies across populations but has no effect on the richness of associated microbial communities

Marília Palumbo Gaiarsa[1,2,3], Sandra Rehan[4], Matthew A. Barbour[1], Quinn S. McFrederick[3]

1. Department of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland
2. University of California, Merced, School of Natural Sciences, 5200 North Lake Road, Merced, CA 95343, USA
3. Department of Entomology, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA
4. Department of Biology, York University, 4700 Keele St, Toronto, ON M3J 1P3, Canada

ORCID:

Marília Palumbo Gaiarsa: https://orcid.org/0000-0003-4414-472X
Sandra Rehan: https://orcid.org/0000-0002-6441-5155
Matthew A. Barbour: https://orcid.org/0000-0001-7686-0400
Quinn S. McFrederick: https://orcid.org/0000-0003-0740-6954

Manuscript submitted as a Note

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

Number of words in the text: 3,089

Elements in the manuscript: Abstract, Introduction, Methods, Results, Discussion, References, Figures, Online Supplement.

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 the bee *Ceratina australensis* from three different wild 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.

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 non-resource 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.

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 affects 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 are composed of over 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 (H1) whether *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 (H3) if dietary breadth and the richness of microbial communities are associated.

Methods

Study sites and data collection

We reanalyzed data previously published in (McFrederick and Rehan 2019) from nests of *Ceratina australensis* collected in January 2015. We analyzed 87 pollen provisions from 38 nests from *Ceratina australensis* from three different populations in Australia (Oppenheimer et al. 2018):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 one 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 of two females 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 in McFrederick and Rehan (2019) (see Online Supplement for details). We only considered 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; Online Supplement). 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 index (*PS*) (Schoener 1968; Roughgarden 1979; Zaccarelli et al. 2013) of each *i* individual (Online Supplement). 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 (Online Supplement). The PS_i index ranges from 0 to 1, 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 9999 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 amplicon sequence variants (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 if 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 centrality, 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 if 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 in 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, and without rarefying the data; Online Supplement). We performed all analyses in R (version 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 <0.001; Fig. 1, Figs. S4-6), and across populations (ANOVA: $F_{2,35} = 14.09, p<0.001$; Fig. 1 & Fig. S5) (H1). The Victoria population was the most specialized, followed by South Australia, and

Queensland (Figs. 1, S4-6). 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 and Tables S1-S2). For example, after accounting for site-level differences in bacterial richness ($F_{2,28} = 5.64, p = 0.009$), there were no clear main or site-dependent effects of specialization (PS_i; $F_{1,28} = 1.52, p = 0.227$, Fig. S8; Site*$PS_i$: $F_{2,28} = 0.61, p = 0.551$). Similarly, for fungal richness we found clear differences among sites ($F_{2,26} = 6.18, p = 0.006$), but no clear main or site-dependent effects of specialization (PS_i: $F_{1,26} = 0.010, p = 0.920$, Fig. S8; Site*$PS_i$: $F_{2,26} = 0.34, p = 0.716$). While it is generally difficult to conclude that a biological factor has no effect, our estimates of uncertainty (SE and 95% CI) 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 and S10, Tables S3-S4). For example, after accounting for the effects of site and degree (and site*degree, Tables S3-S4), there was no evidence of main or site-dependent effects of centrality on bacterial richness (Centrality $F_{1,25} = 0.91, p = 0.349$; Site*Centrality $F_{2,25} = 0.20, p = 0.817$), and this result was consistent regardless of rarefaction method (Fig. S10 and Table S3; all $p > 0.210$). 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: Non-rarefied $p = 0.002$; High read depth $p = 0.062$; Low read depth $p = 0.011$). Specifically, we found consistent positive effects of centrality on fungal richness in the Victoria population (Figs. 2 and 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 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 dataset 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 individual resource sharing (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 transmission 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 bee's 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 (Trøjelsgaard 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 that we 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.

Acknowledgments

We would like to thank Ana Paula A. Assis, Magda Argueta-Guzman, Gustavo Burin, Matthew C. Hutchinson, and Jessica Gillung for their thoughtful discussion and comments. We also thank two anonymous reviewers for their comments.

Funding: Support for this work was provided by the University of California Chancellor's Postdoctoral Fellowship from UC Riverside to MPG, US Department of Agriculture NIFA Hatch funds (CA-R-ENT-5109-H) to QSM and (NC1173) to SMR, National Science Foundation MSB-ECA award #1638728 and DEB-SBS award #1929572 to QSM and National Science Foundation IOS-Behavior #1456296 to SMR, as well as National Geographic (9659-15) to SMR. This project has also received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 888339 to MPG.

Statement of Authorship

MPG conceptualized the study and organized the data. SR collected the data. QSM performed molecular analysis and bioinformatics. MPG and MB analyzed the data and interpreted the results, with input from QSM and SR. MPG 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 Accessibility

All code used in the analyses is available on Github (https://github.com/Magaiarsa/ceratinaIndDiet) and Dryad (https://doi.org/10.5061/dryad.5dv41ns7s). Previously published sequence data are publicly available under NCBI/EMBL/DDBJ accession numbers 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.

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, et al. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & 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: Biological Sciences 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. The 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. Fernando Cagua, C. Coux, and D. Matthias 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. interaction 1(0.2413793).

Douglas, A. E. 2018. The Drosophila model for microbiome research. Lab Animal. Lab animal 47:157–164.

Dupont, Y. L., K. Trøjelsgaard, M. Hagen, M. V. Henriksen, J. M. Olesen, N. M. E. Pedersen, and W. Daniel Kissling. 2014. Spatial structure of an individual-based plant-pollinator network. Oikos 123:1301–1310.

Dupont, Y. L., K. Trøjelsgaard, 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 & 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: Biological Sciences, 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 United States of America 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. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274:303–313.

Klostermeyer, E. C., and H. S. Gerber. 1969. Nesting Behavior of Megachile rotundata (Hymenoptera: Megachilidae) Monitored with an Event Recorder1. 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 United States of America 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.

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.

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, Austria.

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 Publishing.

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.

Trøjelsgaard, 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. Trøjelsgaard, J. M. Olesen, and A. Traveset. 2014. Downscaling pollen-transport networks to the level of individuals. The Journal of animal Ecology 83:306–317.

Ushio, M., E. Yamasaki, H. Takasu, A. J. Nagano, S. Fujinaga, M. N. Honjo, M. Ikemoto, et al. 2015. Microbial communities on flower surfaces act as signatures of pollinator visitation. Scientific Reports 5:8695.

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, et al. 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. Henrik Nilsson, K.-H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, T. Pennanen, et al. (2010). The unite database for molecular identification of fungi–recent updates and future perspectives. New Phytologist 186 (2), 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 (3).

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 (7), 581–583.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, et al. (2010). Qiime allows analysis of high-throughput community sequencing data. Nature methods 7 (5), 335–336.

Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, 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 (D1), 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 (2), e90234.

Figure Legends

Figure 1. Dietary specialization (*PSi*) 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.

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 panel, $\beta = 305$, SE = 125.5, $t_{16} = 2.43$, $p = 0.027$). The right panel shows the plants (same size, white nodes) and bee-individual (gray nodes) interaction network of the Victoria population. Bee individuals on the right panel are scaled according to their weighted closeness centrality. For visualization purposes, three bee individuals are highlighted with colors and shapes matching individuals across panels.

Figure 1

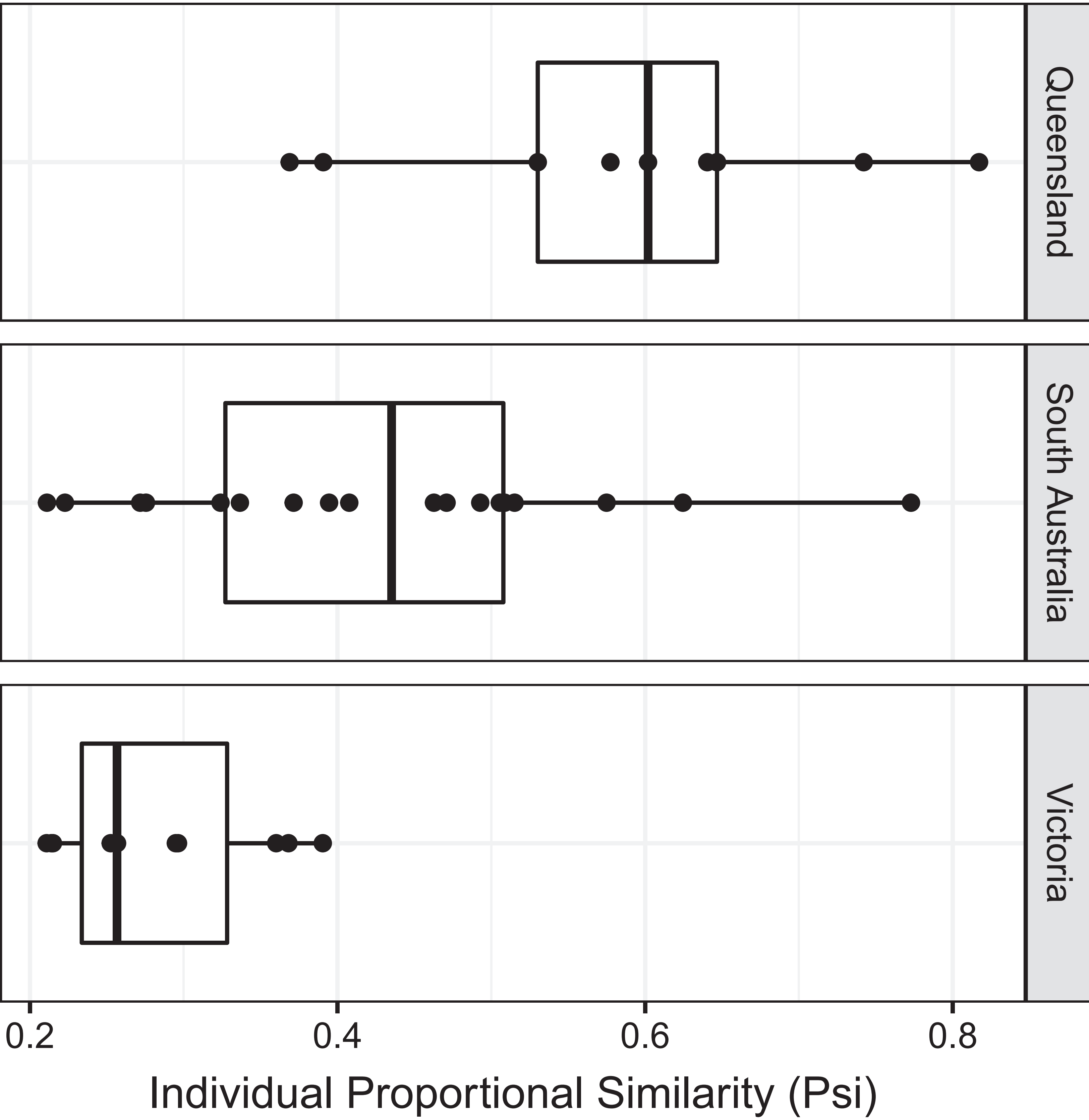
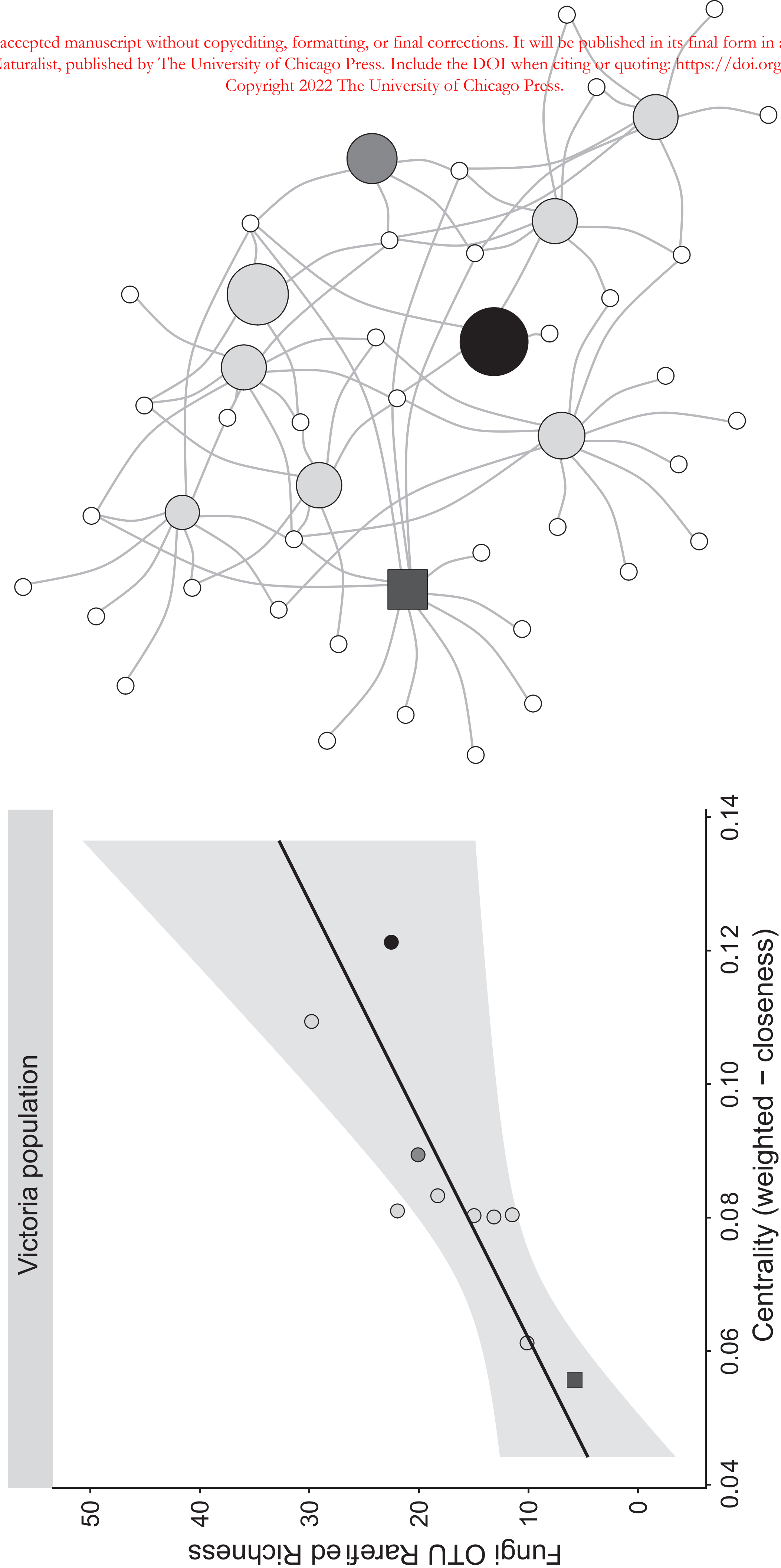

Queensland
South Australia
Victoria
0.2
0.4
0.6
0.8
Individual Proportional Similarity (Psi)

Figure 2


Victoria population
Fungi OTU Rarefied Richness
0
10
20
30
40
50
Centrality (weighted – closeness)
0.04
0.06
0.08
0.10
0.12
0.14

Online Supplement

Individual dietary specialization in a generalist bee varies across populations but has no effect on the richness of associated microbial communities

The American Naturalist

Marília P. Gaiarsa[1,2,3*]

Sandra Rehan[4]

Matthew A. Barbour[1]

Quinn S. McFrederick[3]

1. Department of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland; 2. University of California, Merced, School of Natural Sciences, 5200 North Lake Road, Merced, CA 95343, USA; 3. Department of Entomology, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA 4. Department of Biology, York University, 4700 Keele St, Toronto, ON M3J 1P3, Canada

∗ Corresponding author; e-mail: gaiarsa.mp@gmail.com.

Calculating individual variation

This index considers the proportion of an individual's diet item in comparison to the proportion of that item in the population as a whole: $PS_i = 1 - 0.5 \sum_j |p_{ij} - q_j|$. Thus, the proportional-similarity index of individual i, PS_i, is calculated based on $p_i j$, the proportion of category j in the diet of individual i (or the frequency of plant j in the bee individual i), and q_j, the proportion of category j in the population as a whole (Zaccarelli et al., 2013).

Molecular Analyses and Bioinformatics

Nests were collected at dawn or dusk and were kept chilled before being processed McFrederick and Rehan (2019). They were then split lengthwise and the contents were transferred to sterile cryovials with sterilized forceps. All samples were kept in liquid nitrogen before being transferred to a $-80C$ freezer for storage until DNA extraction. DNA was extracted from each of the 87 pollen provisions using protocols previously described in McFrederick and Rehan (2019).

Briefly, first we prepared separate metabarcoding amplicon libraries for bacteria (the 16S rRNA gene [16S]), fungi (the internal transcribed spacer [ITS]), and plants (RuBisCO large subunit [rbcl]). For 16S and rbcl, we used previously described primers and previously described PCR protocols (McFrederick and Rehan, 2016, 2019), while for fungi we used ITS1f and ITS2 primers (Smith and Peay, 2014). We then used DNA metabarcoding to characterize and quantify the plants, fungi, and bacteria present in each pollen provision. We used QIIME2 (Caporaso et al., 2010) to analyse metabarcoding data and DADA2 (Callahan et al., 2016) to bin reads into exact sequence variants (ESV). For plants (rbcl), we used a database that was recently compiled for metabarcoding studies of pollen (Bell et al., 2017) and the RDP classifier (Cole et al., 2009). Briefly, for bacteria data (16S rRNA) we trained the SILVA 128 database (Quast et al., 2012)to the section of the 16S rRNA gene that is amplified with the primers used, while for the fungi data (ITS) we used the UNITE database (Abarenkov et al., 2010). We identified plants at the genus level, and used ESVs to conduct analysis for the bacteria and fungi.

We included in the analysis data from 38 different individual nests. Each nest had between one and five pollen provisions ($mean = 2.23, sd = 1.06$). Plant diversity varied across sites, with Victoria presenting the highest generic richness (37 genera), followed by Queensland (24 genera), and South Australia (11 genera) (Fig. S1). These results were robust despite the small sample sizes (Figs. S2 and S3).

The effect of sample size

We conducted rarefaction analyses to explore the properties of the different populations. By subsampling the data without replacement in each population it is possible to evaluate how the number of individuals sampled affected the plant species richness. Despite the variation in the number of individuals sampled in each population, the diversity of plant species in the diet of *Ceratina*, per population, is representative. Even though South Australia has the largest number of individuals, if we were to randomly select nine individuals, plant richness would be similar to that of the 18 individuals sampled.

We also explored how mean specialization (*Psi*) changed when we randomly subsampled nine individuals from the South Australia and Victoria populations. For each population, we randomly selected nine individuals 10.000 times, and calculated the mean *Psi* of the population. We found that the average distribution of the subsampled population is similar to the average of the whole population.

The effect of different read depths

We also explored the robustness of our results to different read depths: including all samples regardless of the read depth ("NA"), following the read depths from the original paper ("High"; bacteria = 153, fungi = 530; McFrederick and Rehan 2019), or using a read depth that enables us to include 90% of the data ("Low"; bacteria = 95, fungi = 96). Results were consistent regardless of read depth (Figs. S9 and S11).

The effect of taxonomic resolution on specialization

We tested the effect of the taxonomic resolution of plant data at the genus level on the characterization of specialization in resource use and related results. To do that, we used the Amplicon Sequence Variant (ASV) data to characterize plant interactions, which the same "taxonomic unit" used to access the richness of fungi and bacteria. This is the finest level of taxonomic resolution possible given our data and a proxy for species-level or possibly even strain-level diversity in this study. We found that proportional similarity index (PS_i) using genus and ASV was highly correlated ($r = 0.99, p < 0.001$), which strongly suggest that taxonomic resolution does not impact our results. Similar to results presented in the main text, we found that bee individuals had clear differences in resource use both within (PSi null model: all $p - values < 0.001$), and across populations (ANOVA: $F_{2,35} = 15.97$, $p < 0.001$), and the Victoria population was the most specialized (mean $PS_i = 0.262$, sd=0.06), followed by South Australia (mean $PS_i = 0.419$, sd=0.14), and Queensland (mean $PS_i = 0.590$, sd=0.153).

As for the effect of specialization on microbial richness (H2), similar to the main text analysis we found little evidence for this hypothesis. Here we focus on the results following the read depths from the original paper ("High"; bacteria = 153, fungi = 530; McFrederick and Rehan 2019), but as in the main paper, results were qualitatively similar regardless of read depth used.

For example, after accounting for site-level differences in bacterial richness ($F_{2,28} = 4.13, p = 0.029$), there were no clear main or site-dependent effects of specialization (PSi; $F_{1,28} = 0.19, p = 0.66$; Site*PSi: $F_{2,28} = 1.65, p = 0.21$). Likewise, for fungal richness we found clear differences among sites ($F_{2,26} = 2.93, p = 0.078$), but no clear main or site-dependent effects of specialization ($F_{1,26} = 0.04, p = 0.83$; Site*PSi: $F_{2,26} = 0.24, p = 0.79$).

For the relationship between centrality and microbial richness we also found qualitatively similar results to those presented in the main text. After accounting for the effects of site and degree and for the interaction between site and degree, we found no evidence of main or site-dependent effects of centrality on bacterial richness (Centrality $F_{1,25} = 3.29, p = 0.08$ Site*Centrality $F_{2,25} = 0.22, p = 0.79$). 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 (Non-rarefied $p = 0.015$; High read depth $p = 0.17$; Low read depth $p = 0.002$). Once again, we found consistent positive effects of centrality on fungal richness in the Victoria population whereas there was no consistent evidence of centrality effects on fungal richness at the other sites.

Supplementary Figures and Tables

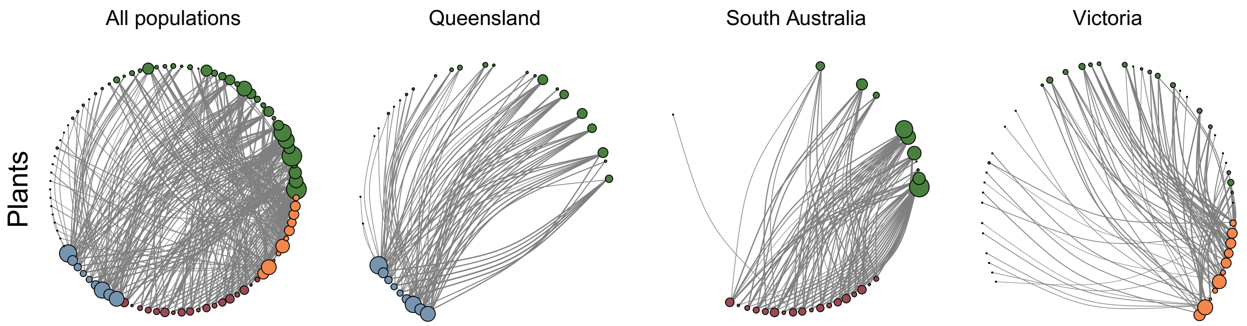


Figure S1: Interaction network between plants genera (in green) and the different *Ceratina australiensis* individuals of each population: Queensland (blue), South Australia (red), and Victoria (orange). The wider the circle, the greater the number of plants an individual bee interacts with. The position of the nodes (circles) is fixed to facilitate comparison across populations.

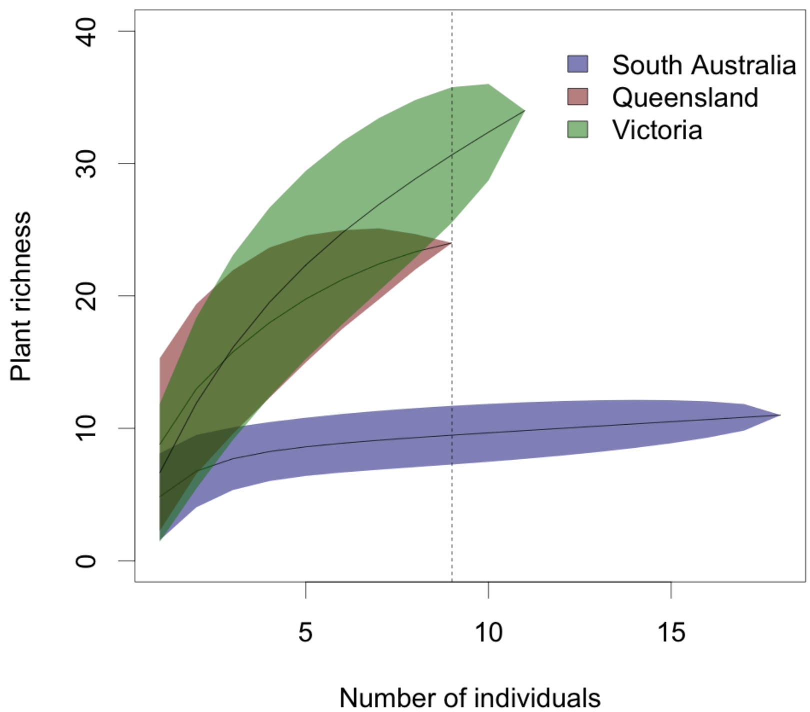


Figure S2: Rarefaction curve of the number of plant genera found as individuals are added to the samples.

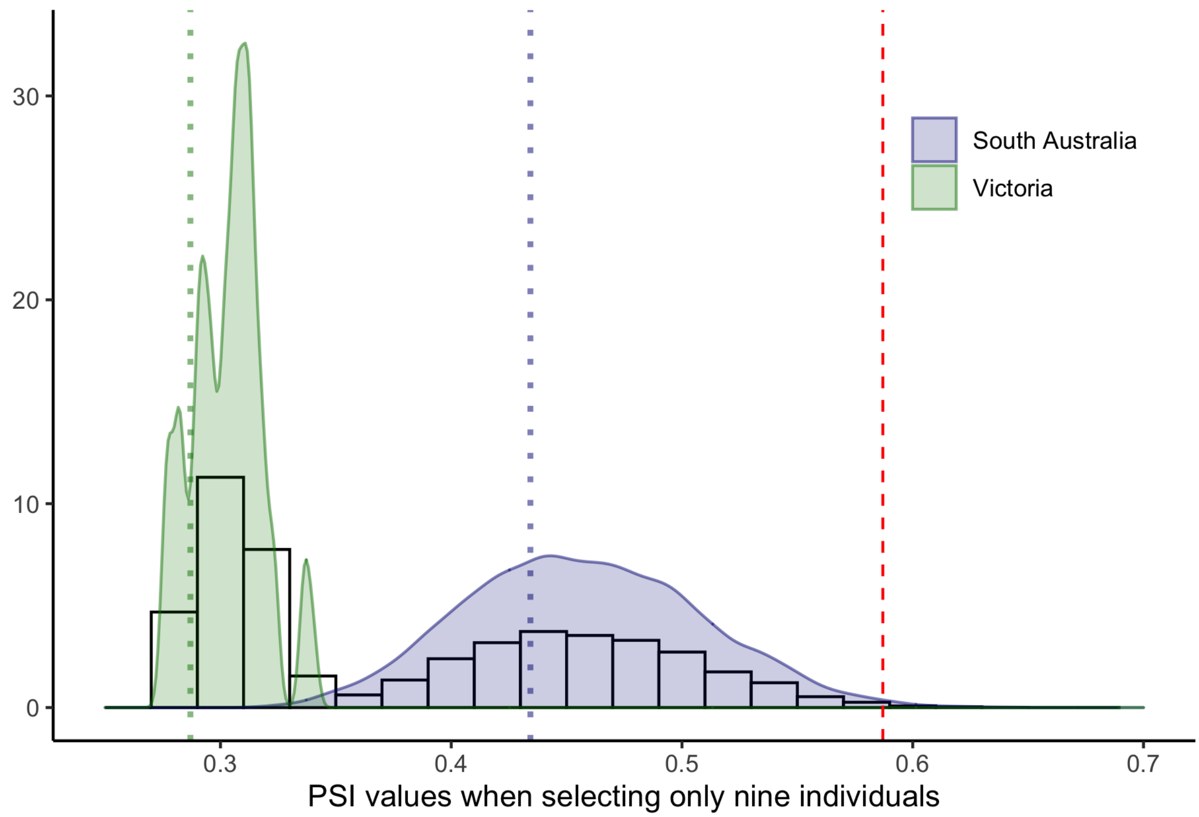


Figure S3: Distribution of mean specialization (PS_i) when randomly subsampling nine individuals from the South Australia (blue) and Victoria (green) populations. Dotted lines indicate the population average Psi when including all individuals and the dashed red line indicates the average PS_i of the Queensland population.

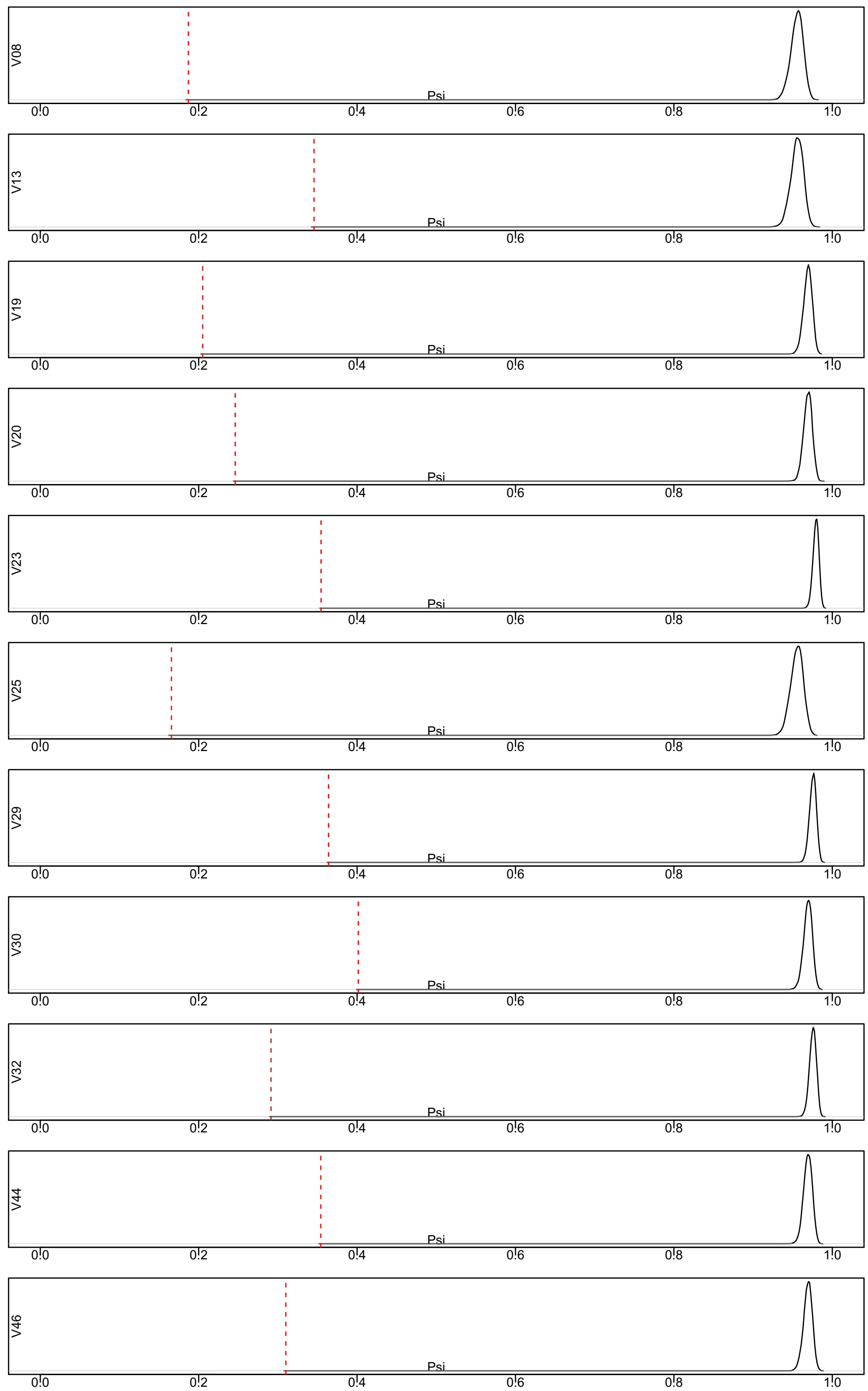


Figure S4: Variation in individual proportional similarity among individuals of the Victoria population. The red bar represents the empirical value and in black is the frequency distribution of mean bootstrapped values across 9999 simulations.

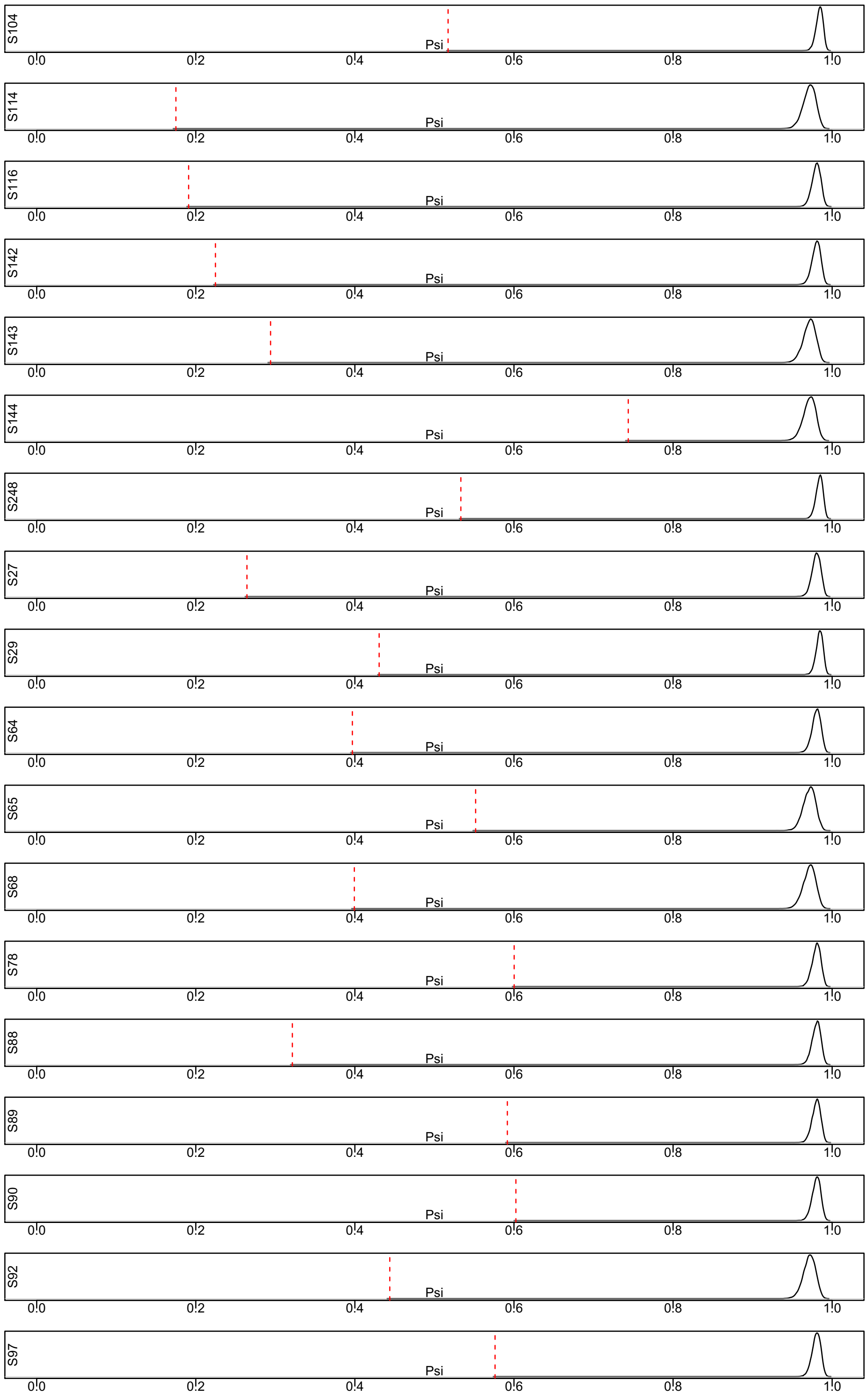


Figure S5: Variation in individual proportional similarity among individuals of the South Australia population. The red bar represents the empirical value and in black is the frequency distribution of mean bootstrapped values across 9999 simulations.

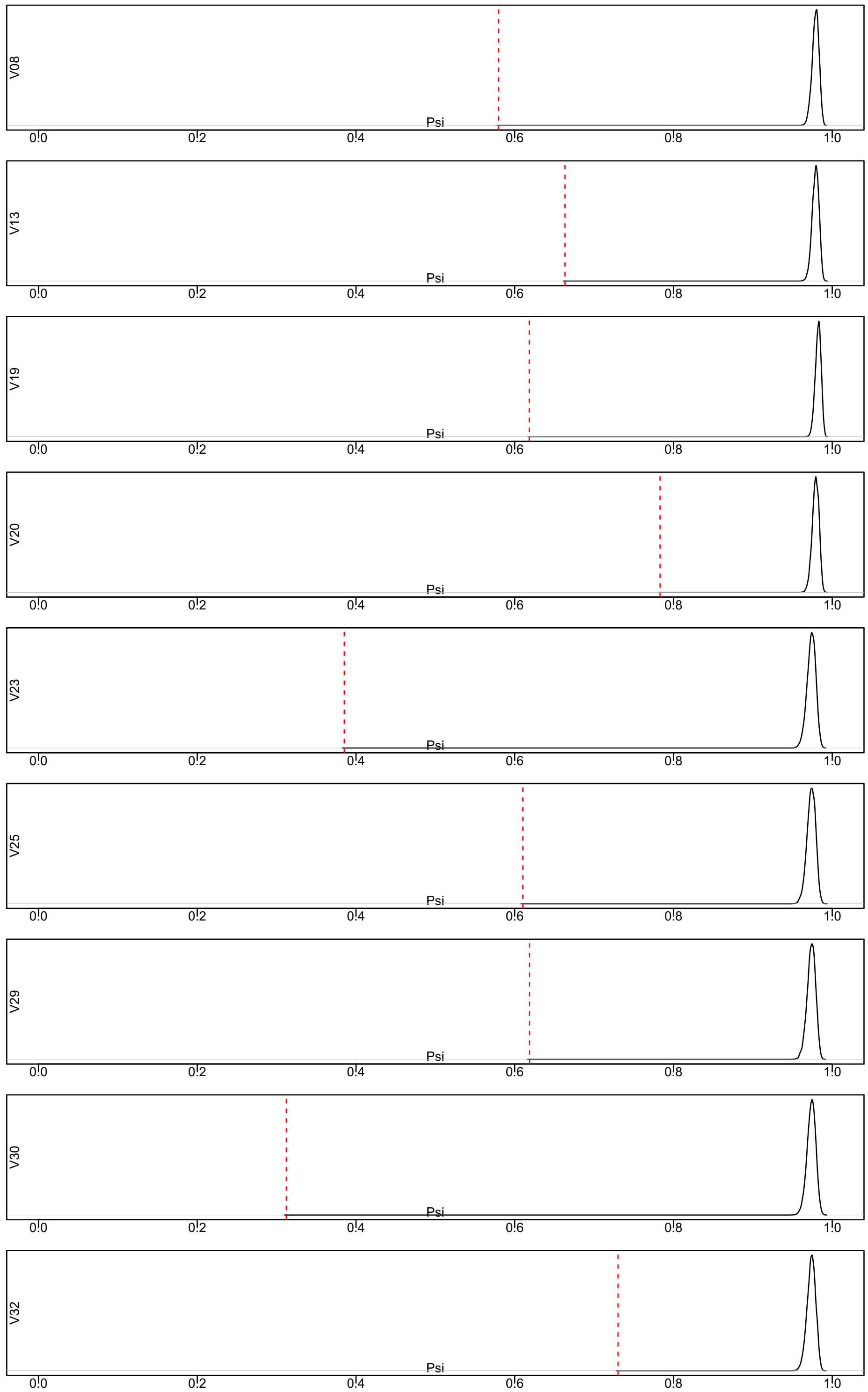


Figure S6: Variation in individual proportional similarity among individuals of the Queensland population. The red bar represents the empirical value and in black is the frequency distribution of mean bootstrapped values across 9999 simulations.

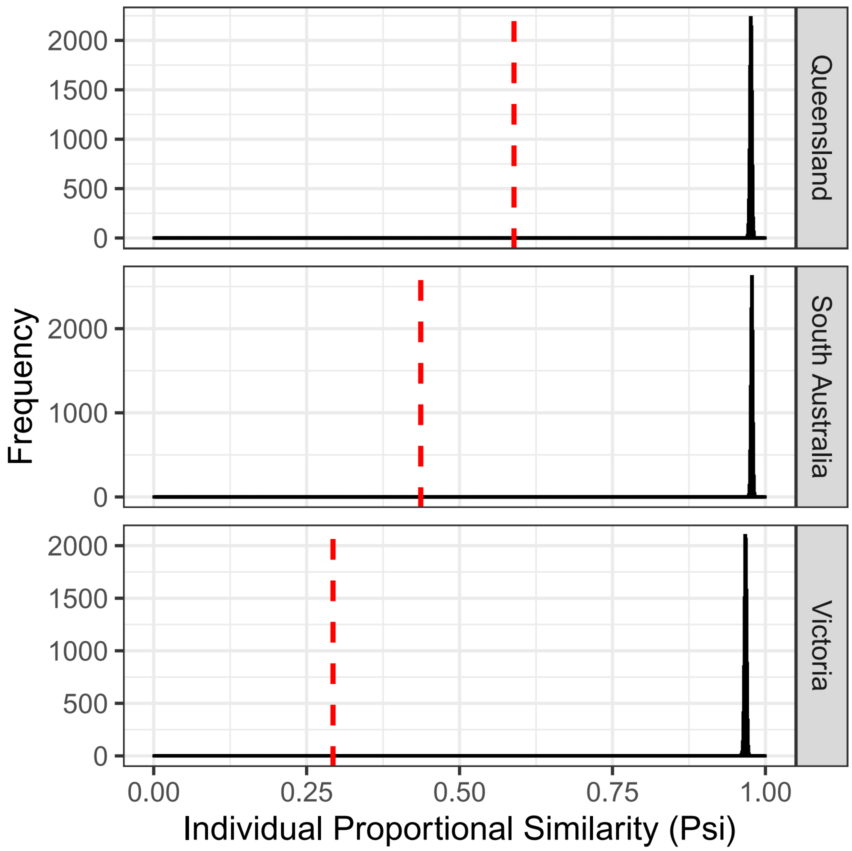


Figure S7: Frequency distribution of mean PSi values for each population. Red lines represent the empirical PSi mean of each population, and the frequency distribution of the PSi values of the null models. All populations presented empirical values much smaller than expected the a null distribution.

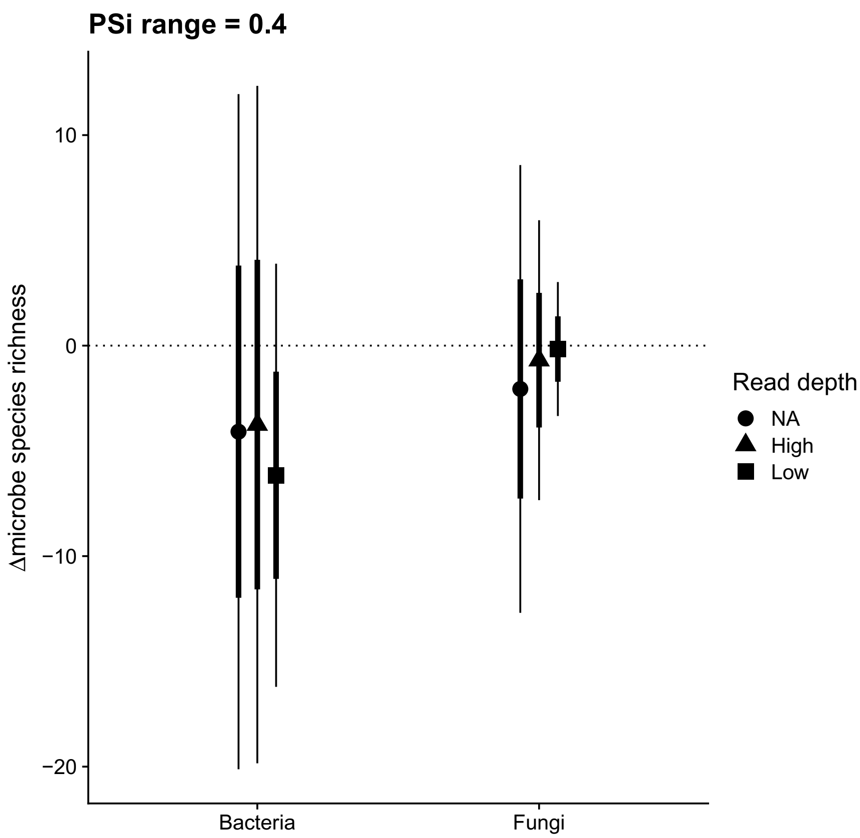


Figure S8: Biological effect of specialization (PS_i) on microbial species richness after controlling for the effect of sampling site. Effect size corresponds to the predicted change in microbial richness with an increase in specialization of 0.4units, which reflects the average range of specialization within individuals of each population. Points correspond to mean estimates, while thick and thin lines correspond to standard error and 95% confidence intervals, respectively. Different point shapes correspond to different read depths the data were rarefied to. Note that high and low read depths differ for bacteria (high = 153, low = 95) and fungi (high = 530, low = 96).

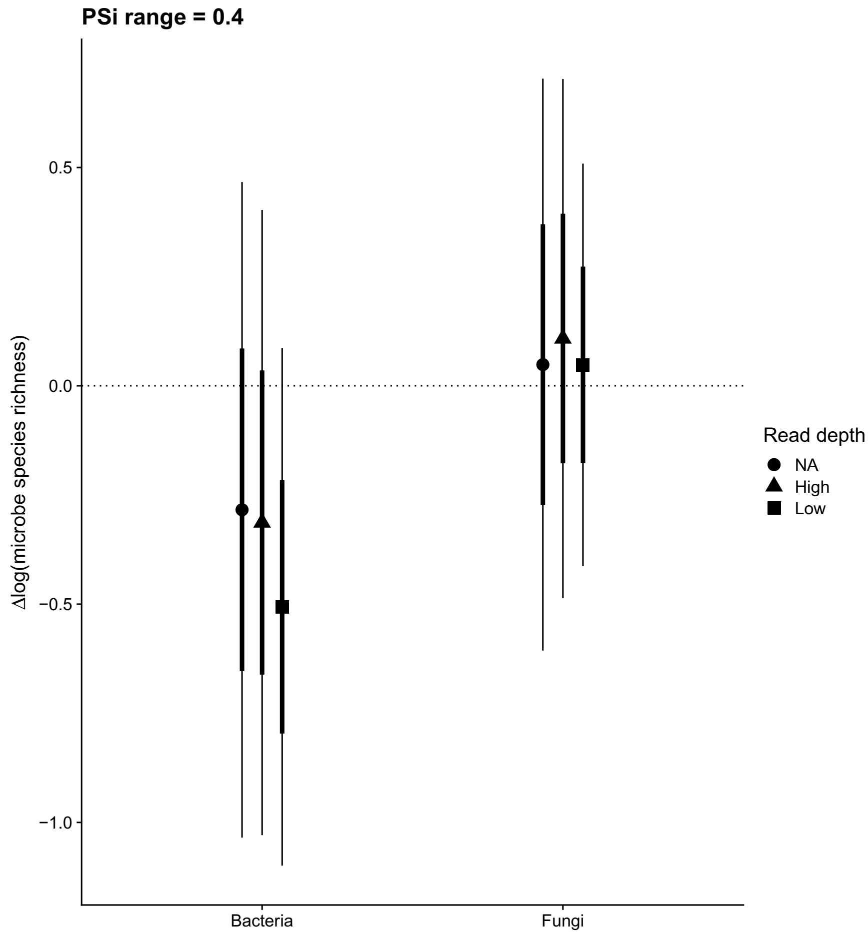


Figure S9: Biological effect of specialization (PS_i) on log of microbial species richness after controlling for the effect of sampling site. Effect size corresponds to the predicted change in microbial richness with an increase in specialization of 0.4units, which reflects the average range of specialization within individuals of each population. Points correspond to mean estimates, while thick and thin lines correspond to standard error and 95% confidence intervals, respectively. Different point shapes correspond to different read depths the data were rarefied to. Note that high and low read depths differ for bacteria (high = 153, low = 95) and fungi (high = 530, low = 96).

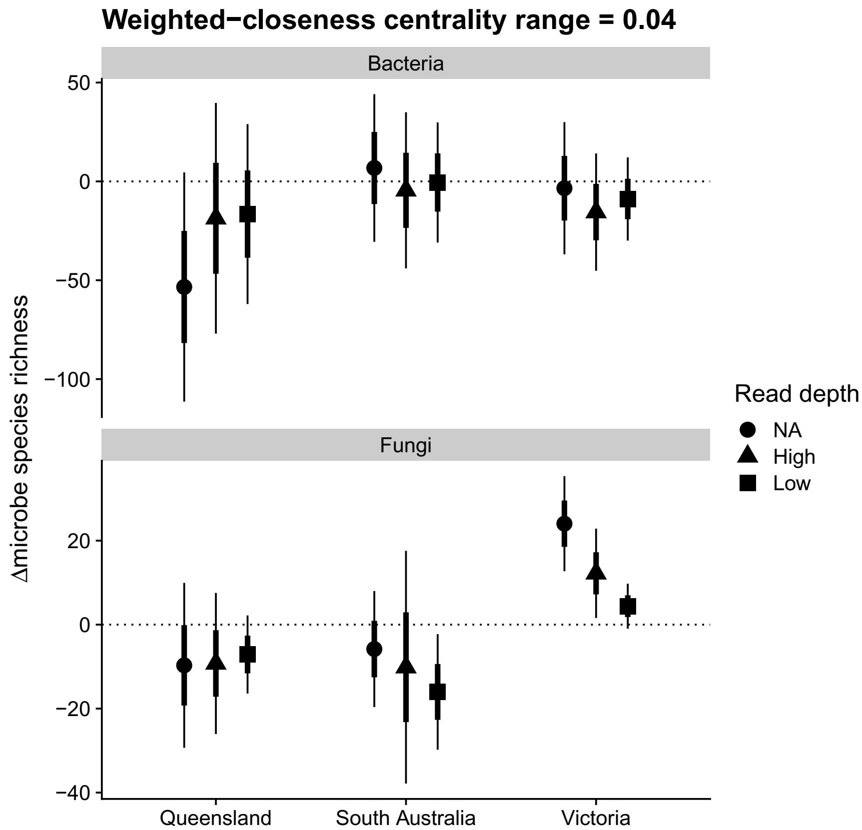


Figure S10: Biological effect of centrality on microbial species richness after controlling for the effect of sampling site and degree. Effect size corresponds to the predicted change in microbial richness with an increase in centrality of 0.04 units, which reflects the average range of centrality among individuals of each population. Points correspond to mean estimates, while thick and thin lines correspond to standard error and 95% confidence intervals, respectively. Different point shapes correspond to different read depths the data were rarefied to. Note that high and low read depths differ for bacteria (high = 153, low = 95) and fungi (high = 530, low = 96).

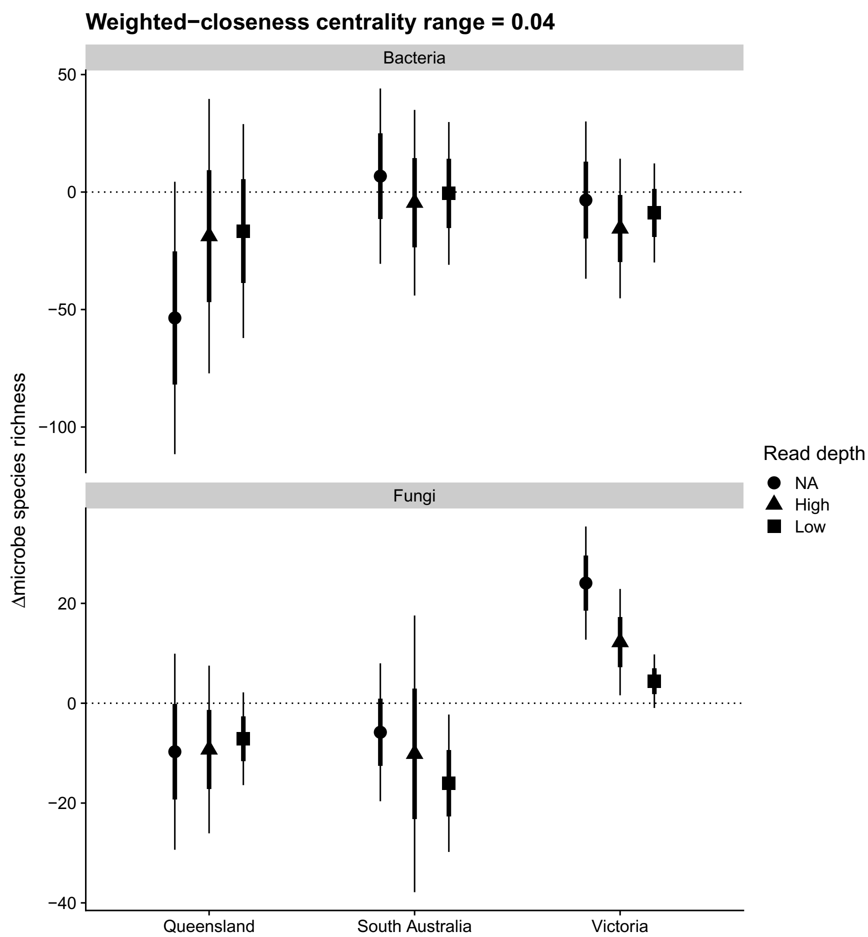


Figure S11: Biological effect of centrality on log microbial species richness after controlling for the effect of sampling site and degree. Effect size corresponds to the predicted change in microbial richness with an increase in centrality of 0.04 units, which reflects the average range of centrality among individuals of each population. Points correspond to mean estimates, while thick and thin lines correspond to standard error and 95% confidence intervals, respectively. Different point shapes correspond to different read depths the data were rarefied to. Note that high and low read depths differ for bacteria (high = 153, low = 95) and fungi (high = 530, low = 96).

Table S1: Sequential ANOVA (Type I) of site and bee specialization (PS_i) effects on bacterial richness.

Source	df	SS	MS	F	P
No rarefaction (NA)					
Site	2	1333.4	666.72	2.86	0.072
PS_i	1	60.8	60.81	0.26	0.613
Site × PS_i	2	249.8	124.92	0.54	0.590
Residuals	32	7451.7	232.87		
Rarefy to 153 reads (High)					
Site	2	1021.01	510.50	4.05	0.031
PS_i	1	29.72	29.72	0.24	0.632
Site × PS_i	2	339.25	169.62	1.35	0.279
Residuals	24	3025.43	126.06		
Rarefy to 95 reads (Low)					
Site	2	881.40	440.70	5.64	0.009
PS_i	1	119.26	119.26	1.53	0.227
Site × PS_i	2	95.14	47.57	0.61	0.551
Residuals	28	2189.15	78.18		

Table S2: Sequential ANOVA (Type I) of site and bee specialization (PS_i) effects on fungal richness.

Source	df	SS	MS	F	P
No rarefaction (NA)					
Site	2	627.12	313.56	6.64	0.004
PS_i	1	2.71	2.71	0.06	0.812
Site × PS_i	2	8.81	4.40	0.09	0.911
Residuals	30	1415.67	47.19		
Rarefy to 530 reads (High)					
Site	2	149.58	74.79	2.94	0.077
PS_i	1	1.11	1.11	0.04	0.837
Site × PS_i	2	14.42	7.21	0.28	0.756
Residuals	19	483.10	25.43		
Rarefy to 96 reads (Low)					
Site	2	105.15	52.58	6.18	0.006
PS_i	1	0.09	0.09	0.01	0.920
Site × PS_i	2	5.77	2.88	0.34	0.716
Residuals	26	221.15	8.51		

Table S3: Sequential ANOVA (Type I) of site, bee degree, and bee weighted-closeness centrality effects on bacterial richness.

Source	df	SS	MS	F	P
No rarefaction (NA)					
Site	2	1333.4	666.72	2.86	0.073
Degree	1	12.0	11.95	0.05	0.822
Site × Degree	2	132.3	66.15	0.28	0.755
Centrality	1	101.3	101.32	0.44	0.515
Site × Centrality	2	767.2	383.60	1.65	0.210
Residuals	29	6749.6	232.74		
Rarefy to 153 reads (High)					
Site	2	1021.01	510.50	3.48	0.049
Degree	1	32.76	32.76	0.22	0.642
Site × Degree	2	31.43	15.71	0.11	0.899
Centrality	1	207.40	207.40	1.41	0.248
Site × Centrality	2	39.59	19.79	0.13	0.875
Residuals	21	3083.21	146.82		
Rarefy to 95 reads (Low)					
Site	2	881.40	440.70	4.84	0.017
Degree	1	6.87	6.87	0.08	0.786
Site × Degree	2	2.04	1.02	0.01	0.989
Centrality	1	82.95	82.95	0.91	0.349
Site × Centrality	2	37.09	18.54	0.20	0.817
Residuals	25	2274.59	90.98		

Table S4: Sequential ANOVA (Type I) of site, bee degree, and bee weighted-closeness centrality effects on fungal richness.

Source	df	SS	MS	*F*	*P*
No rarefaction (NA)					
Site	2	627.12	313.56	11.82	<0.001
Degree	1	108.72	108.72	4.10	0.053
Site × Degree	2	52.42	26.21	0.99	0.385
Centrality	1	124.06	124.06	4.68	0.040
Site × Centrality	2	425.98	212.99	8.03	0.002
Residuals	27	716.00	26.52		
Rarefy to 530 reads (High)					
Site	2	149.58	74.79	4.11	0.036
Degree	1	46.89	46.89	2.58	0.128
Site × Degree	2	17.34	8.67	0.48	0.629
Centrality	1	22.89	22.89	1.26	0.278
Site × Centrality	2	120.54	60.27	3.31	0.062
Residuals	16	290.97	18.19		
Rarefy to 96 reads (Low)					
Site	2	105.15	52.58	8.99	0.001
Degree	1	15.48	15.48	2.65	0.117
Site × Degree	2	11.65	5.83	1.00	0.384
Centrality	1	0.09	0.09	0.02	0.900
Site × Centrality	2	65.30	32.65	5.58	0.011
Residuals	23	134.48	5.85		

References

Abarenkov, K., R. Henrik Nilsson, K.-H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, T. Pennanen, et al. (2010). The unite database for molecular identification of fungi–recent updates and future perspectives. *New Phytologist 186*(2), 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*(3).

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*(7), 581–583.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, et al. (2010). Qiime allows analysis of high-throughput community sequencing data. *Nature methods 7*(5), 335–336.

Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, et al. (2009). The ribosomal database project: improved alignments and new tools for rrna analysis. *Nucleic acids research 37*(suppl_1), D141–D145.

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*(10), 2302–2311.

McFrederick, Q. S. and S. M. Rehan (2019). Wild bee pollen usage and microbial communities co-vary across landscapes. *Microbial ecology 77*(2), 513–522.

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*(D1), 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*(2), e90234.

Zaccarelli, N., D. I. Bolnick, and G. Mancinelli (2013). Ri n s p: an r package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution 4*(11), 1018–1023.