Microbial Ecology (2023) 85:330–334
https://doi.org/10.1007/s00248-021-01922-5

NOTE

Higher Variability in Fungi Compared to Bacteria in the Foraging Honey Bee Gut

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Received: 4 February 2021 / Accepted: 8 November 2021 / Published online: 8 January 2022

Abstract

Along with bacteria, fungi can represent a significant component of animal- and plant-associated microbial communities. However, we have only begun to describe these fungi, much less examine their effects on most animals and plants. Bacteria associated with the honey bee, *Apis mellifera*, have been well characterized across different regions of the gut. The mid- and hindgut of foraging bees house a deterministic set of core species that affect host health, whereas the crop, or the honey stomach, harbors a more diverse set of bacteria that is highly variable in composition among individual bees. Whether this contrast between the two regions of the gut also applies to fungi remains unclear despite their potential influence on host health. In honey bees caught foraging at four sites across the San Francisco Peninsula of California, we found that fungi were less distinct in species composition between the crop and the mid- and hindgut than bacteria. Unlike bacteria, fungi varied substantially in species composition throughout the honey bee gut, and much of this variation could be predicted by the location where we collected the bees. These observations suggest that fungi may be transient passengers and unimportant as gut symbionts. However, our findings also indicate that honey bees could be vectors of infectious plant diseases as many of the fungi we found in the honey bee gut are recognized as plant pathogens.

Keywords *Apis mellifera* · Beta diversity · Gut microbiota · Pathogens · Symbionts

Recently, the honey bee, *Apis mellifera,* has emerged as a model system for uncovering rules that govern the assembly of host-associated microbial communities and their effects on host health [1, 2]. Studies on honey bee microbes suggest that different regions of the gut house distinct microbial communities, making it necessary to examine these communities separately in order to understand how they affect host health [3, 4]. For example, in the mid- and hindgut (hereafter the intestine, Fig. 1a), a deterministic set of functionally indispensable core microbes represent the majority of the microbial cells inhabiting the bee gut [3, 5, 6]. These core species are found across all healthy workers regardless of location [5]. In contrast, the crop, or the honey stomach, shows high heterogeneity in microbial species composition even among healthy workers, likely reflecting the spatial and temporal variation of ingested environmental microbes [7–10]. However, research on the honey bee gut microbiota has focused almost exclusively on bacteria, and it remains unknown whether the contrast between crop and intestinal communities applies only to bacteria or is also observed in other groups of microbes, such as fungi, which may affect host health in ways that are currently underappreciated [11, 12].

In this study, we examined both bacteria and fungi in foraging workers to test two hypotheses: (1) fungal species composition is as distinct between the crop and the intestine as is bacterial species composition and (2) fungi, like

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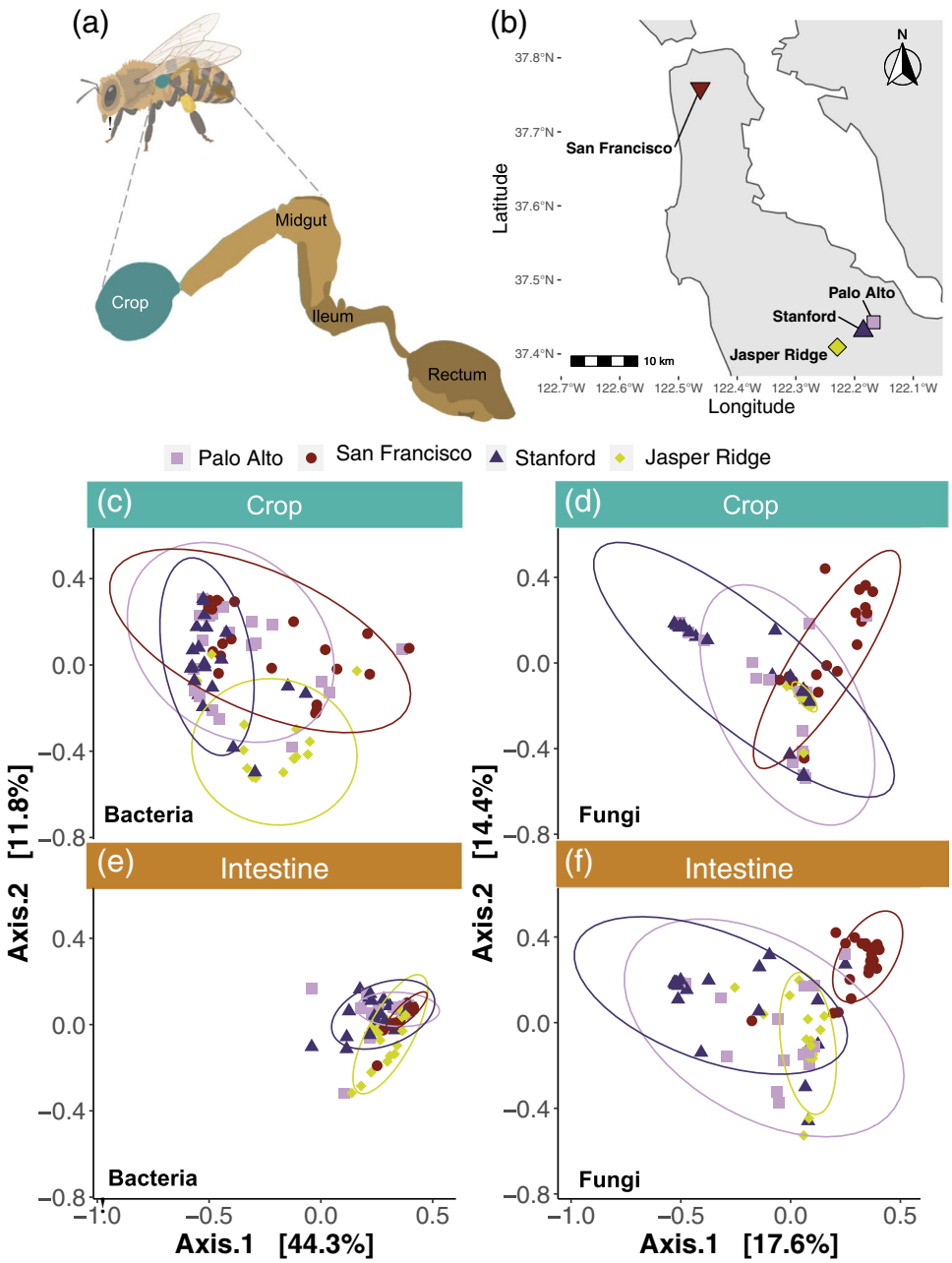

Fig. 1 **a** Honey bee gut anatomy color-coded by sampling scheme dividing the gut into the crop (turquoise) and the remaining posterior intestine regions (gold). **b** Map of the collection sites along the San Francisco Peninsula: Jasper Ridge Biological Preserve ($N=25$ bees), Stanford University ($N=26$ bees), Palo Alto ($N=24$ bees), and San Francisco ($N=26$ bees). **c–f** Bacterial communities were relatively consistent across sampling sites, whereas fungal communities were more reflective of collection site. Principle coordinate analyses (PCoA) based on Bray–Curtis dissimilarity matrices calculated from rarefied OTU tables show variation in **c** bacterial crop communities, **d** fungal crop communities, **e** bacterial intestine communities, and **f** fungal intestine communities. Each point represents a bee individual, and shape and color indicate collection site

bacteria, show more variable species composition in the crop than in the intestine. To test these hypotheses, we collected a total of 101 *A. mellifera* foraging workers at four sites on the San Francisco Peninsula in CA, USA (Fig. 1b, Table S1). We dissected the entire gut, separating the crop from the intestine. We then extracted and sequenced the bacterial V4 region of the 16S ribosomal RNA gene (505–806) and the fungal ITS1-5.8S-ITS2 region [13] (see Supplementary Information). Sequences were clustered into operational taxonomic unites (OTUs) using VSEARCH [14] and taxonomy assigned for bacterial and fungal OTUs using QIIME [15] and UNITE [16].

As expected, bacterial community composition was most strongly predicted by gut region (PERMANOVA, gut region: $R^2=0.34$, $p=0.001$, Fig. 1c–f, Fig. 2a), and higher among-host variation was detected in the crop than in the intestine (beta deviation [17]: $F_{1,168}=14.5$, $p=0.0002$). Although fungi also showed high variability in the crop (Figs. 1 and 2, beta deviation: $F_{3,161}=40.8$, $p<0.0001$, Fig. S2), fungi in the intestine were more diverse in species composition (Shannon, tissue: $F_{1,164}=6.56$, $p=0.01$, Fig. S3). Additionally, fungi retained more of the among-site differences from the crop to the intestine than did bacteria (Figs. 1 and 2). Sample site was the strongest predictor of fungal species

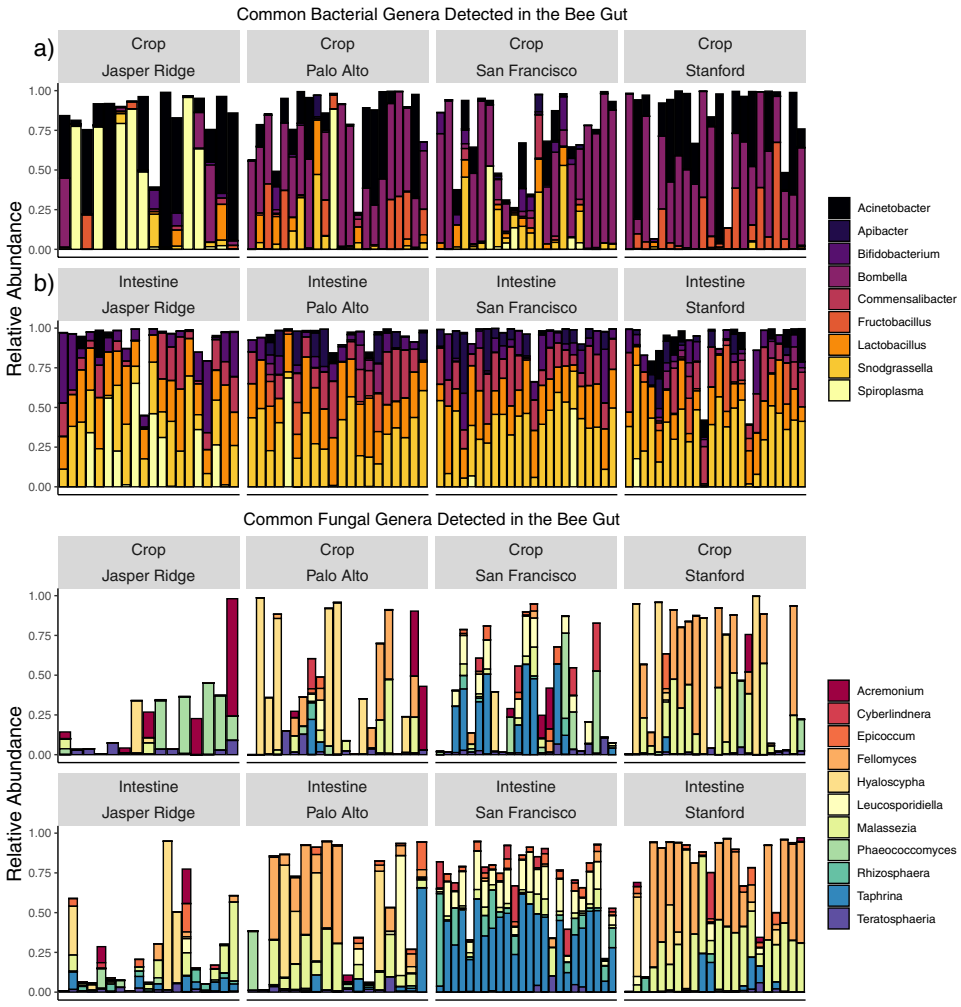

Fig. 2 Relative abundance of dominant (> 2,000 reads across all samples) microbial genera across sites and gut regions within each bee individual illustrating high variation within crops. **a** Dominant bacterial genera, including *Snodgrassella, Bombella, Acinetobacter, Lactobacillus, Commensalibacter,* and *Bifidobacterium*. Each bar represents one sample and the relative abundance of bacterial genera out of the total reads detected in that sample. **b** Dominant fungal genera, including *Fellomyces, Malassezia, Taphrina, Hyaloscypha, Leucosporidiella, and Phaeococcomyces*. Each bar represents one sample and the relative abundance of fungal genera out of the total reads detected in that sample

composition not just in the crop, but also in the intestine (PERMANOVA, site: $R^2 = 0.14$, $p = 0.001$, Fig. 1 d and f, Fig. 2b), with gut region explaining only a small proportion of fungal composition (PERMANOVA, gut region: site, $R^2 = 0.03$, $p = 0.001$). Bray–Curtis distance, which is calculated based on relative OTU abundance, and Jaccard distance, based on OTU presence/absence, showed qualitatively identical results (Figs. 1 and S1).

To further quantify differences between bacteria and fungi, we applied the CLAMtest, a multinomial species classification method [18], which differentiated OTUs into four categories: generalist, crop-associated, intestine-associated, and too rare to classify. We found that only 1.5% of bacterial OTUs were categorized as generalists, whereas 7.3% of fungal OTUs fell into this category (Fig. 3a–d). Furthermore, only 1.8% of bacterial OTUs were classified as intestine-associated, whereas 11.4% of fungal OTUs were classified as intestine-associated.

We examined how tightly crop composition was correlated with intestinal composition by applying Mantel tests to bacterial and fungal data separately. Crop composition was positively correlated with intestinal composition in both bacteria (Mantel $r = 0.34$, $p < 0.0001$, Fig. 3e) and fungi (Mantel $r = 0.24$, $p \leq 0.0001$, Fig. 3f). However, quantile regression analysis indicated that bacteria in the intestine were correlated with those in the crop similarly across all three quantiles (10th slope = 0.11, 50th slope = 0.24, 90th slope = 0.31, Fig. 3e), whereas the slope of the relationship for fungi depended on the quantile examined (10th slope = 0.78, 50th slope = 0.42, 90th slope = 0.08, Fig. 3f).

Taken together, our results reject both of the hypotheses we set out to test, highlighting contrasting compositional patterns between bacteria and fungi in the honey bee gut. Specifically, we found that honey bees retained more of the across-site differences from the crop to the intestine in fungi than in bacteria. Furthermore, unlike the constrained set of bacterial species in the intestine [3], fungal species composition was highly variable not just in the crop, but also in the intestine. The broad distribution of fungal taxa we found throughout the gut suggests that these microbes are ingested from external sources, with some of them opportunistically colonizing the gut [19]. Our Mantel test results

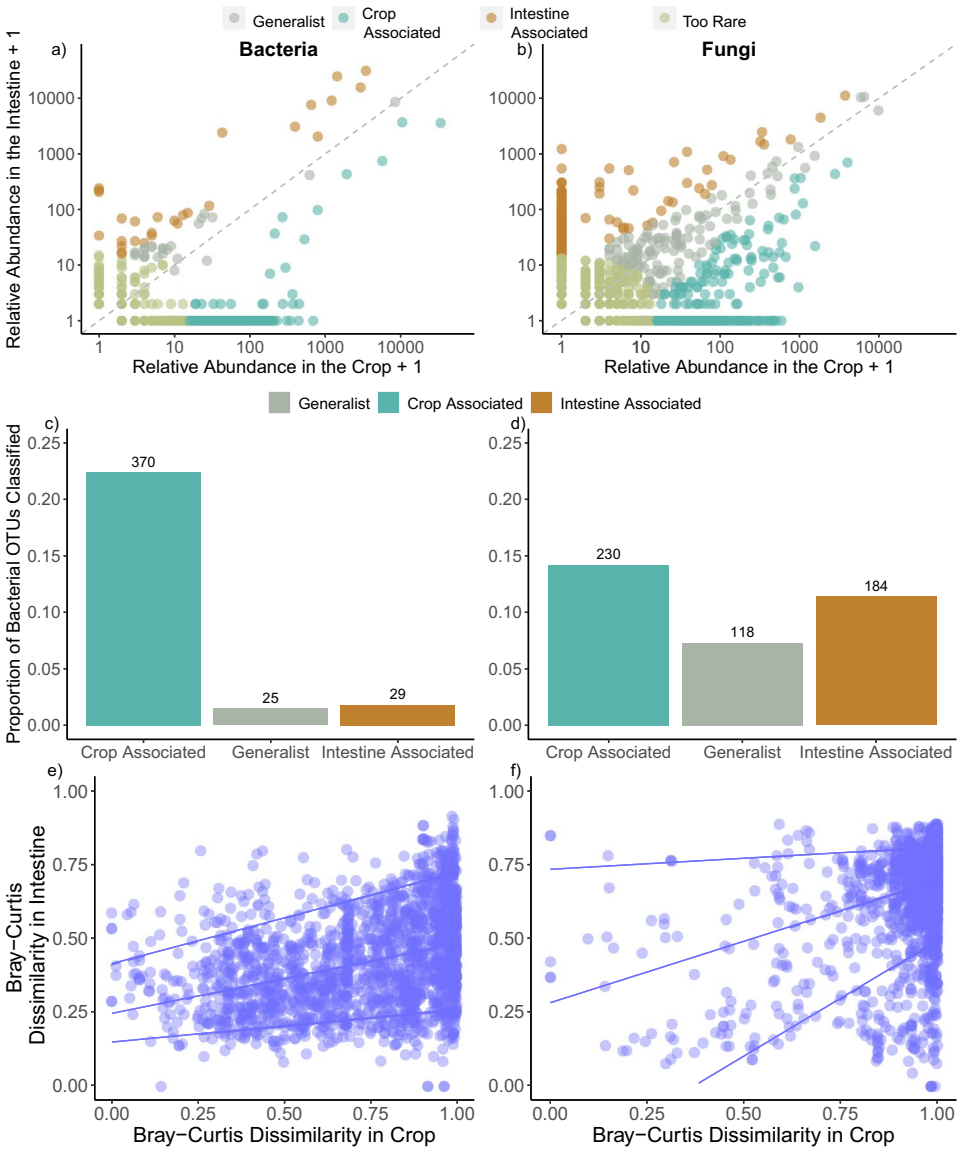

Fig. 3 Fewer bacterial OTUs were categorized as intestine-associated and generalists compared to fungi using a multinomial species classification method (clamtest) to sort OTUs into categories based on relative abundance in each domain: **a** bacteria and **b** fungi. Summarized classification results for **c** bacteria and **d** fungi. Relationships between **e** bacterial crop and intestine community structure and **f** fungal crop and intestine community structure. Lines are regressions against the 10th, 50th, and 90th quantiles, respectively, from bottom to top. Changes in slope reflect the extent to which the composition of intestine communities depends on crop communities

further indicate that various fungal taxa disappear in a seemingly stochastic fashion as they move from the crop to the intestine, perhaps because fungi are low in absolute abundance throughout the gut. These processes inferred for fungi contrast the deterministic filtering of bacteria from the crop to the intestine that has been documented previously and corroborated here [3, 5].

Some of the 20 most common fungal OTUs that could be identified with moderate certainty in our study were reported previously as plant pathogens, including *Taphrina carpin* and *Exidia glandulosa* (Table S3). Assuming that some fungi remain viable as they pass through the gut [20], our study supports the role of honey bees as vectors of a diversity of plant fungal pathogens. Transmission of phytopathogens on the surface of honey bees has been implicated in the spread of bacterial and fungal pathogens [21, 22], but the extent to which fecal transmission of fungal pathogens contributes to plant epidemics remains unknown. Honey bee hives are often transported among multiple orchards and farms for pollination [23]. Our data indicate that the composition of gut fungal communities is specific to foraging sites. However, if honey bees do act as vectors of plant-pathogenic fungi, fungal pathogens that would otherwise be locally restricted could be transmitted more broadly when hives are transported. It is also possible that some of the fungal taxa we identified are pathogens to bees and other arthropods [24], including *Aspergillus* (Table S3), which can cause stonebrood in honey bees [25].

In summary, here we provide evidence that fungal species composition is not as distinct between the crop and intestine as in bacteria and that fungal species composition is highly variable across the entire gut, unlike bacteria. These findings suggest that most fungi found in the honey bee gut may be transient passengers rather than symbionts that affect the

health of the host. In future research, quantification of absolute microbial abundance paired with manipulative studies testing the efficacy of honey bees of vectors of phytopathogens is needed to determine the ecological relevance of this transience to plant disease transmission and pollination.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-021-01922-5.

Acknowledgements We thank Megan Morris for advice on DNA extraction, sequencing, and data analysis. We also thank Callie Chappell, Nick Hendershot, Jesse Miller, Chih-Fu Yeh, and two anonymous reviewers for comments.

Author Contribution LED, PAS, CED, MLW, and TF designed the study, and LED, PAS, CED, and MLW collected samples. LED and CG analyzed data, and LED wrote the first draft of the manuscript. All authors contributed to editing the manuscript.

Funding This work was funded by NSF (DEB 1737758). CED was supported by the Stanford Summer Research Program.

Data Availability Raw sequence data are openly available in the Sequence Read Archives of the National Center for Biotechnology Information (NCBI), accession number: PRJNA775827. URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA775827

Code Availability Upon acceptance, code will be made available on Dryad Digital Repository.

Declarations

Conflict of Interest The authors declare no competing interests.

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