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Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota

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Summary

Bumblebees are ecologically and economically important as pollinators of crop and wild plants, especially in temperate systems. Species, such as the buff-tailed bumblebee (*Bombus terrestris*), are reared commercially to pollinate high value crops. Their highly specific gut microbiota, characterised by low diversity, may affect nutrition and immunity and are likely to be important for fitness and colony health. However, little is known about how environmental factors affect bacterial community structure. We analyzed the gut microbiota from three groups of worker bumblebees (*B. terrestris*) from distinct colonies that varied in rearing and foraging characteristics: commercially reared with restricted foraging (RR); commercially reared with outside foraging (RF); and wild-caught workers (W). Contrary to previous studies, which indicate that bacterial communities are highly conserved across workers, we found that RF individuals had an intermediate community structure compared to RR and W types. Further, this was shaped by differences in the abundances of common OTUs and the diversity of rare OTUs present which we propose results from an increase in the variety of carbohydrates obtained through foraging.

Introduction

Insects and other pollinators provide a vital ecosystem service to 87.5% of the world's plant species (Ollerton et al., 2011) and demand for pollination services in crops is high (estimated global value of €153 billion; Gallai et al., 2009). As a consequence, there is an increasing awareness of the ecological and economic importance of such organisms. However, whilst demand for pollination services continues to rapidly increase, there is growing evidence for declines in pollinator populations (Biesmeijer et al., 2006; vanEngelsdorp et al., 2008; Aizen and Harder, 2009; Potts et al., 2010a; Potts et al., 2010b). Declines are likely driven by multiple factors including disease, pesticide use, host plant loss and changes in land management (Cameron et al., 2011; Dicks et al., 2013; Scheper et al., 2014). A link between the reduction of plant pollination, and a drop in pollinator diversity and abundance is also well established (Memmott et al., 2004; Biesmeijer et al., 2006; Albrecht et al., 2012). An increasing human population will only intensify demands on wild and managed pollinator populations to meet future food security needs (Klein et al., 2007; Aizen et al., 2008).

In temperate systems, eusocial bumblebees (*Bombus* spp.) are important and prolific plant pollinators. Some species are commercially managed to pollinate high value glasshouse and fruit crops (Klein et al., 2007; Leonhardt and Blüthgen, 2012). This practice is increasingly common, with between 30,000-60,000 bumblebee colonies per year being imported into the UK alone (Lye et al., 2011). Ensuring the production of healthy bumblebee colonies will be vital to sustain the growing demand for their services (Pettis et al., 2012). There is therefore interest in how commercially reared bees may differ from wild types in terms of physiology, and how interactions between them may affect fitness (Otterstatter and Thomson, 2008).

The insect gut is known to harbour a microbial community which is thought to aid host fitness through enhanced nutrition, immunity and colony health (Dillon and Dillon, 2004; Warnecke et al., 2007; Cariveau et al., 2014; Pernice et al., 2014). Recent studies suggest the *Bombus* gut bacterial community is predominately comprised of members from: Orbaceae (Gammaproteobacteria), Lactobacillaceae (Firmicutes), Neisseriaceae (Betaproteobacteria), Acetobacteraceae (Alphaproteobacteria), Bacteroidetes and Actinobacteria (Koch and Schmid-Hempel, 2012; Koch et al., 2013; Kwong and Moran, 2013; Cariveau et al., 2014). While much of the evidence suggests that

69 the gut microbiota of bumblebees are highly conserved and of relatively low diversity (Koch and
70 Schmid-Hempel, 2011b; Martinson et al., 2011) it has been shown that detectable shifts in bumblebee
71 gut bacterial diversity may occur in response to infection (Koch et al., 2012; Cariveau et al., 2014).
72 How other environmental changes affect gut microbial community structure remains unexplored.
73
74 Here, we utilized 16S rRNA gene targeted next generation sequencing techniques to analyze the gut
75 microbiota from three groups of individual adult female bumblebees (*Bombus terrestris*) from distinct
76 colonies that were: commercially reared with no outside (restricted) foraging (RR, $n = 6$), commercially
77 reared but released for outside foraging (RF, $n = 10$) and field-caught workers collected from
78 Buckinghamshire and the Isle of Wight, UK (W, $n = 7$). Given the low diversity and highly specific
79 microbiota reported previously, we adopted a null hypothesis that diversity and composition of *B. t.*
80 *audax* host gut microbiota would not be influenced by rearing and foraging conditions. The current
81 study aimed to establish whether gut microbiota responded to host foraging, i.e. does a commercially
82 reared host, with controlled food resources (within colony standardised pollen and nectar solution)
83 have a detectably different gut microbiota from that of wild populations.
84
85
86

Results and discussion

Bacterial diversity and composition from whole gut samples was assessed using 16S rRNA gene targeted high-throughput sequencing. From 23 bee gut samples, a total of 2,465,708 sequence reads (mean ± SD per sample, 107204.7± 59212.6) were included in the final analysis and 373 distinct operational taxonomic units (OTUs) identified. The average numbers of bacterial sequence reads per sample were similar among the three groups: commercially reared but restricted to colony (RR), 96,484 ± 55,741 (*n* = 6); commercially reared but with outside foraging (RF), 100,533 ± 53,812 (*n* = 10); and wild-caught workers (W), 125,924 ± 64,867 (*n* = 7). The number of OTUs we identified is higher than that in studies applying traditional culture independent techniques - ranging from 9 to 146 sequenced OTUs (Koch and Schmid-Hempel, 2011b; Martinson et al., 2011).Thus, the increased sampling depth through the application of next generation sequencing (NGS) appears to have captured more of the inherent gut microbial diversity. When compared to other insects guts (e.g up to 726 OTUs were identified in the termite hind gut alone, Köhler et al., 2012), an overarching richness of 373 OTUs is relatively low, although comparable to that of the honey bee (Moran et al., 2012), suggesting that the bumblebee gut microbiome does indeed represent a low diversity, specialized community.

It is expected that a microbial metacommunity would display a positive relationship between frequency and abundance of individual taxa (OTUs) from within its constituent communities (van der Gast et al., 2011). Consistent with this prediction, the abundance of individual bacterial OTUs, across all samples (Figure 1a), was significantly correlated with the number of individual gut sample communities that they occupied. Separating component taxa within a host microbiota into common and rare groupings reveals important aspects of taxa-abundance distributions (van der Gast et al., 2011; van der Gast et al., 2014). Here, we partitioned the OTUs into 'common' (defined as those present in the upper quartile of sample occupancy with >75% across all samples) and 'rare' groupings. The 28 common OTUs accounted for 97.4% of the total sequence abundance while the rare group comprised the majority of the diversity (345 'rare' OTUs). Similarly, Cariveau et al. (2014) determined that high abundance OTUs represented 98.9% of sequences from *B. bimaculatus* and *B. impatiens* gut microbiota samples.

Mean OTU richness in the whole microbiota was significantly higher within the RF group (121.5 ± 10.4, mean ± SD) when compared to the other samples (RR, 97.2 ± 18.7; and W, 83.0 ± 2.1; Figure 1b and Table S1). The same significant pattern was reflected in the rare microbiota (RR, 71.2 ± 18.1; RF, 96.8 ± 9.8; and W, 57.4 ± 2.0), but not in the common microbiota which did not significantly differ between groups (RR, 26.0 ± 1.2; RF, 24.7 ± 1.11; and W, 25.6 ± 0.8; Figure 1b and Table S1). We therefore assert that observed patterns in richness are driven by compositional changes in the rare microbiota. This was confirmed by pair wise comparisons of turnover rates (number of taxa/OTUs eliminated and replaced; Figure 1c), where whole microbiota turnover between groups followed that of the rare microbiota comparisons. No turnover was observed between the common microbiota (Figure 1c), however the common microbiota did contribute most to patterns of whole microbiota composition (Figure 1d). Bray-Curtis quantitative index similarity (S_{BC}) revealed the whole microbiota to be highly similar to the corresponding common microbiota (mean S_{BC} = 0.99 ± 0.01, n = 3 pair wise comparisons). Conversely, the rare microbiota was highly dissimilar between whole microbiota and corresponding rare microbiota (mean S_{BC} = 0.04 ± 0.03), and were divergent between rare microbiota groups (mean S_{BC} = 0.23 ± 0.15; Figure 1d).

Analysis of the uniqueness and sample group allocation of OTUs (Figure 2) demonstrated that, in addition to the 28 common OTUs, a further 102 OTUs (taxa) were shared across all treatments. These appear to be an integral part of the wild *B. t. audax* gut microbiota, and therefore likely to be retained across generations. Interestingly, when looking at the allocation of rare OTUs the reared foraging group had the highest number unique of OTUs (75) when compared to the other sample group types (RR = 9, W =13). Further, none of the OTUs detected were shared solely between the RR and W groups, suggesting that although gut microbiota from commercially reared populations are distinct from wild populations, when allowed to forage a shift in microbiota from a commercially reared to wild pattern occurs. As such the RF group would represent a population with microbiota in flux, showing a pattern which shares both commercially reared and wild attributes. If this is the case it would be interesting to consider whether the RF gut microbiota population would fully transition to a wild type and how long such a transition would take. Analysis of similarity (ANOSIM) tests give further weight to the patterns observed. While the microbiota (whole, common and rare) from RR and W

146 samples were significantly divergent (Table 1), the RF microbiota shared attributes with both the RR
147 and W groups' microbiota.
148
149 In order to determine which OTUs contributed most to the observed shift in community abundance
150 and composition similarity percentage (SIMPER) analysis was performed (Table 2). Representative
151 OTUs commonly found within insect and hymenopteran guts were prevalent within the bumblebees
152 studied here - including members of the Neisseriaceae, Orbaceae, Enterobacteriaceae,
153 Lactobacillaceae, Pseudomonadaceae and Bifidobacteriaceae (Kosako et al., 1984; Babendreier et
154 al., 2007; Novakova et al., 2009; Killer et al., 2010; Wilkes et al., 2011; Koch et al., 2013; Duron,
155 2014; Engel et al., 2014; Killer et al., 2014b; Killer et al., 2014a). Two common microbiota group
156 OTUs, identified as *Snodgrassella alvi* and *Gilliamella apicola,* contributed the most to the dissimilarity
157 between groups. Both have previously been found to be dominant members within honeybees and
158 other bumblebee species (Koch and Schmid-Hempel, 2011a; Kwong and Moran, 2013). *S. alvi* had a
159 higher relative abundance in the RR samples (52.1%) than both the RF (29.5%) and W (22.4%)
160 samples. Conversely, *G. apicola* was more abundant in the wild samples (30.9%) than the reared (RR
161 = 22.9% and RF = 17.9%).
162
163 Analysis of the genomes of these organisms has suggested that they perform complementary roles
164 within the bee gut. Kwong et al. (2014b) suggest that *G. apicola* is a saccharolytic fermenter,
165 possessing the genes for pathways associated with carbohydrate metabolism, whereas *S.alvi* shows
166 no evidence of these, instead possessing pathways involved in the metabolism of carboxylates. It
167 appears that increases in *G. apicola* mean relative abundance in the wild bees represents a biological
168 response to increased foraging (i.e, a wide range of pollen and nectar types) which contrasts with
169 commercially reared bees, fed upon a single nectar source and restricted (irradiated) pollen. This is
170 further supported by the presence of other OTUs which exhibited increases in relative abundances
171 related to foraging. The common OTU identified as *Arsenophonus nasoniae* demonstrated an
172 increase in abundance in favour of foraging ability (RR=0.02%, RF=6.1%, and W= 15.8%, Table 2). A
173 genomic study based upon *Arsenophonus nasoniae* indicated that this species contains intact
174 pathways for carbohydrate metabolism (Darby et al., 2010). A common OTU identified as
175 *Fructobacillus* also increased with foraging (RR = 0.02%, RF = 0.29%, W = 12.5%). The genus

176 *Fructobacillus* is a group of fructophilic lactic acid bacteria that prefer fructose as a growth substrate
177 and inhabit fructose-rich habitats, including bumble (Koch and Schmid-Hempel, 2011b) and honey
178 bee guts (Endo and Salminen, 2013). Interestingly, there appeared to be role differences occurring
179 within related taxa. Members of the *Lactobacillus* genus are able to metabolise multiple carbohydrate
180 types (Killer et al., 2014a; Kwong et al., 2014a); here a common OTU identified as *Lactobacillus*
181 *kunkeei* increased in relative abundance with the ability to forage, whereas another common and
182 distinct *Lactobacillus* OTU decreased (Table 2). Overall, wild foraging represents an increase in the
183 range and diversity of pollen/nectar sources and therefore the bacteria able to process these
184 additional carbohydrate types.
185
186 Finally, canonical correspondence analysis revealed that variance in microbiota was explained by
187 foraging, rearing and host weight (Table 3 and Figure S1). Undetermined variation could be explained
188 by factors not measured here, for example infection with microbial parasites (e.g. *Crithidia* and *Nosema*)
189 and colony age; both previously associated with differences in *Bombus s*pp. gut communities (Koch et
190 al., 2012; Cariveau et al., 2014).
191
192 In eusocial bees common bacteria are often considered to be synonymous with indigenous/core host
193 gut microbes and are most likely acquired through vertical transmission or within colony interactions
194 (Powell et al., 2014). In contrast, rare/non-core microbiota often contain members which are
195 associated with non-host environments, and are most likely acquired though horizontal transmission
196 (Cariveau et al., 2014). Within our study the rare bacteria shaped observed patterns in diversity. We
197 suggest these detected changes are likely to be through low abundance organisms which have
198 changed in response to host bees foraging on more diverse food resources, in addition to the
199 horizontal acquisition of bacteria from the environment. In a recent study in honey bees it was found
200 that the majority of transmission of gut bacteria was through within hive interactions, rather than
201 environmental exposure (Powell et al., 2014). If this pattern holds true for bumblebees it would
202 suggest that, although the environment does undoubtedly serve as an important and variable
203 reservoir for bacterial immigration, the existing gut microbiota has the capacity to adapt to new
204 foraging resources.
205

206 Overall, we have shown that significant variation in microbiota can result from intraspecific differences
207 in bumblebee rearing and foraging. Given the vital ecosystem services bumblebees provide in
208 pollination of crop and native plants future work should focus on the temporal and functional
209 significance of these shifts in bacterial diversity and composition, and any subsequent effect upon
210 host health and fitness.

211 **Experimental procedures**

212 *Bumblebee samples*

213 Commercially reared (Biobest N.V., Westerlo, Belgium) mature female worker individuals of *Bombus*
214 *terrestris audax* (Table S2) were collected after 26 days into the experiment from distinct colonies that
215 were restricted to colony (RR, *n* = 6) or allowed to forage (RF, *n* = 10) in agricultural land near to the
216 NERC Centre for Ecology & Hydrology, Wallingford, Oxfordshire, UK. Wild female worker individuals
217 (W, *n* = 7) were collected in July 2009 from within agricultural landscapes on the Isle of Wight, UK (*n*
218 = 3), and the Hillesden Estate, Buckinghamshire, UK (*n* = 4) as part of a previous study (Carvell et al.,
219 2012). Molecular microsatellite analysis data were examined, generated from a previous study
220 (Carvell et al., 2012), to minimise probability of processing collected individuals from the same colony.
221 Members of the reared restricted (RR) group were reared within laboratory conditions with a diet
222 consisting of 'Biogluc' (a 66% commercial sugar solution) and fresh (frozen), gamma irradiated pollen,
223 both supplied by Biobest N.V, Belgium. Members of the reared foraged group (RF) were treated
224 identically to the lab reared group until introduction to the wild. At this point - in order to encourage
225 foraging from the local agricultural landscape - no additional nutritional substitute was provided. RR
226 and RF individuals were sampled during July and August 2013.

227

228 *DNA extraction and sequencing*

229 Whole guts from individual specimens (frozen at -80^{o}C within 2 hours of collection) which had been
230 commercially reared or captured in the wild, were used to extract microbiome DNA using the
231 PowerSoil®-htp 96 Well Soil DNA Isolation Kit (Mobio Laboratories Inc., Carlsbad, CA), under the
232 manufacturers recommended protocol. In addition, PCR negative controls consisting of extraction
233 and PCR blanks were also processed and likely kit contaminants removed from analysis (Salter et al.,
234 2014). Approximately 20-30 ng of template DNA was amplified using Q5® high-fidelity DNA
235 polymerase (New England Biolabs, Hitchin, UK) each with a unique golay barcoded primer. After an
236 initial denaturation step at 98 ºC for 2 min, individual PCR reactions employed 25 cycles of an initial
237 30 sec, 98ºC denaturation step, followed by annealing phase for 30 sec at 53ºC, and final extension
238 step lasting 90 secs at 72 ºC. All reactions employed a final extension step of 5 min at 72ºC. Primers
239 based upon the universal primers 27F (5'- CCATCTCATCCCTGCGTGTCTCCGACTCAG) and 338R
240 (5'- GCTGCCTCCCGTAGGAGT) were adapted to include ion torrent linker, golay barcode (Whiteley

et al., 2012) and spacer sequences (Table S2). An amplicon library consisting of ~400 bp amplicons spanning the V1-V2 hypervariable regions of the 16S rRNA gene was generated from gel purified pooled products of 4 replicate PCR reactions, per sample. Quantification was performed on an Agilent 2200 TapeStation system and an equimolar mix of PCR products was prepared and diluted to 20pM in dH_20. This library was sequenced using an Ion Torrent Personal Genome Machine (Life Technologies, Paisley, UK) with a 316 chip.

Sequence analysis

The Mothur sequencing analysis platform was used to analyse the resulting data (Schloss et al., 2009; Schloss et al., 2011). Sequence quality checks included the removal of failed reads, low-quality ends, tags and primers. Further, sequences were aligned against the Mothur SILVA reference bacterial database and any unaligned sequences that included ambiguous base calls and/or homopolymers longer than 8 bases were also eliminated. Finally, chimeras were identified and discarded through Mothur using the UCHIME algorithm (Edgar et al., 2011). The resultant alignment was used to assemble operational taxonomic unit (OTU) clusters at 96% identity, through distance measures (Schloss and Handelsman, 2005, 2006). Taxonomic identity of these OTUs was assigned using the default settings with the mothur RDP reference database. As an additional measure the identity of reference sequences from key OTUs was corroborated using the NCBI's BLASTN program. OTUs identified in negative controls were removed from further analysis (Salter et al., 2014). The raw sequence data reported in this study have been deposited in the European Nucleotide Archive under study accession number ERP007145 and sample accession number ERS557783. The relevant barcode information for each sample is shown in Table S2.

Statistical analysis

Operational taxonomic units (OTUs) were partitioned into common and rare microbiota groups using a modification of the method previously described (van der Gast et al., 2011; van der Gast et al., 2014). Based on a significant positive distribution-abundance relationship, the persistent and abundant common OTUs were defined as those in more than 75% of all samples, while all other OTUs falling outside of the upper quartile were considered to be rare. Richness (S^*) was used as previously described (Rogers et al., 2013). It is known that pair wise comparisons will be affected by large

differences in sample size (Gihring et al., 2012). Therefore, S^* was calculated with a uniform re-sample size (to match the smallest sequence size in each microbiota group [whole, common, and rare]) following 1000 iterations in each instance and performed in R version 3.1.1 (Oksanen et al., 2013; The R Development Core Team, 2013)

Taxa turnover between consecutive samples was measured using the method described by Brown and Kodric-Brown (1977). Turnover was defined as: $t = b + c / S1 + S2$. Where b = the number of OTUs present only in the first sample; c = the number of OTUs present only in the second sample; $S1$ = the total number of OTUs in the first sample; and $S2$ the total number of OTUs in the second sample (Brown and Kodric-Brown, 1977). Two-sample *t*-tests, regression analysis, coefficients of determination (r^2), residuals and significance (P) were calculated using Minitab software (version 16, Minitab, University Park, PA, UK). The Bray-Curtis quantitative index of similarity and subsequent average linkage clustering of community profiles was performed using PAST (Paleontological Statistics, version 3.01) program, available from the University of Oslo (http://folk.uio.no/ohammer/past). Analysis of similarity (ANOSIM) and similarity of percentages analysis (SIMPER) were performed using the PAST (version 3.01). The Bray-Curtis quantitative index of similarity was used as the underpinning community similarity measure for both ANOSIM and SIMPER analyses. Canonical correspondence analysis (CCA) was used to relate the variability in the distribution of microbiota between groups to environmental factors. Environmental variables that significantly explained variation in the gut microbiota were determined with forward selection (999 Monte Carlo permutations; $P<0.05$) and used in CCA. CCA analyses were preformed in PAST (version 3.01) as previously described (Hazard et al., 2013).

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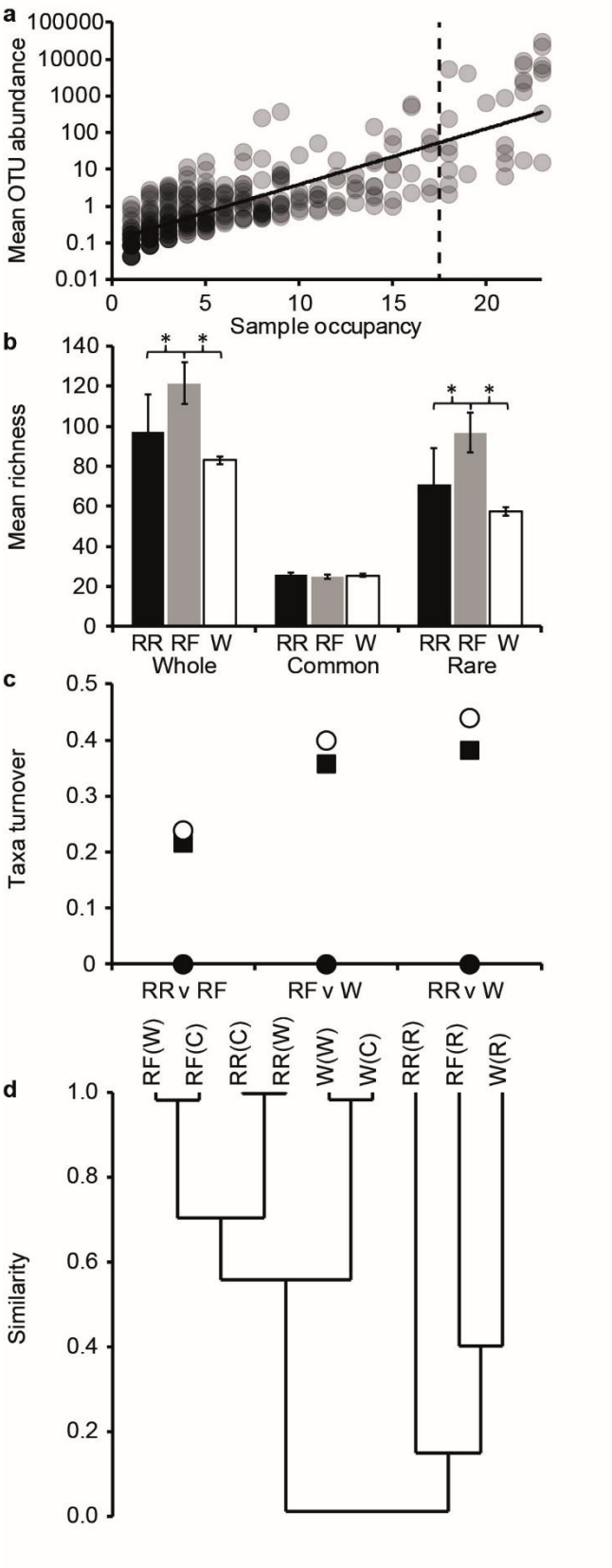
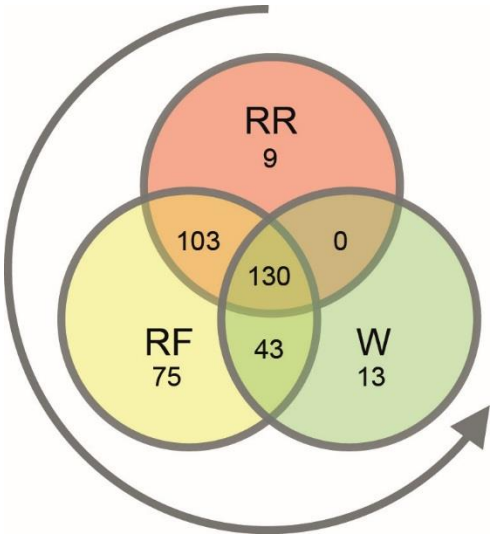
515 **Figure and Table legends**

516 **Figure 1** Comparisons of community characteristics between bee groups. (a) Distribution and
517 abundance of OTUs from bee gut microbiota samples. Given is the number of samples for which
518 each bacterial taxon was observed to occupy, plotted against the mean abundance across all
519 samples (n = 23, r^2 = 0.68, $F_{1, 371}$ = 787.6, P < 0.0001). Common OTUs were defined as those that
520 fell within the upper quartile (dashed lines), and rare OTUs defined as those that did not. (b) Mean
521 OTU richness of whole, common and rare microbiota within the reared restricted (RR), reared foraged
522 (RF) and wild (W) bee groups. Asterisks denote significant differences in comparisons of diversity at
523 the P < 0.05 level determined by two-sample t-tests (t-test summary statistics are given in Table S1).
524 (c) Taxa turnover within whole (solid squares), common (solid circles) and rare (open circles)
525 microbiota between sample groups. (d) Dendrogram of similarity between groups partitioned into the
526 whole (W), common (C) and rare (R) microbiota. Metacommunity profiles were compared using the
527 Bray-Curtis quantitative index of similarity and unweighted pair-group method using arithmetic mean
528 (UPGMA).
529
530 **Figure 2** Unique and shared OTUs between groups. Values given within circles represent, unique
531 OTUs to the reared restricted (RR) group, reared foraged (RF), and wild (W) groups. Values given in
532 overlapping regions correspond to the number of OTUs shared between two given groups. Central
533 overlapping region corresponds to OTUs shared across all group types inclusive of the 28 common
534 OTUs. The arrow represents direction of proposed community transition from commercially reared to
535 wild type microbiota.
536
537
538

Figure 1


a
Mean OTU abundance
100000
10000
1000
100
10
1
0.1
0.01
0
5
10
15
20
Sample occupancy
b
Mean richness
140
120
100
80
60
40
20
0
RR RF W
Whole
RR RF W
Common
RR RF W
Rare
c
Taxa turnover
0.5
0.4
0.3
0.2
0.1
0
RR v RF
RF v W
RR v W
d
Similarity
1.0
0.8
0.6
0.4
0.2
0.0
RF(W)
RF(C)
RR(C)
RR(W)
W(W)
W(C)
RR(R)
RF(R)
W(R)

542 **Figure 2**



543

Table 1 Analysis of similarity (ANOSIM) of whole, common, and rare microbiota between reared restricted (RR), reared foraged (RF), and wild (W) bee groups. ANOSIM test statistic (*R*) and probability (*P*) that two compared groups are significantly different at the $P < 0.05$ level (denoted with asterisks) are given in the lower and upper triangles, respectively. ANOSIM *R* and *P* values were generated using the Bray-Curtis measure of similarity.

Whole	RR	RF	W
RR	-	0.990	0.008*
RF	-0.177	-	0.832
W	0.295	-0.085	-
Common	**RR**	**RF**	**W**
RR	-	0.992	0.009*
RF	-0.177	-	0.869
W	0.298	-0.092	-
Rare	**RR**	**RF**	**W**
RR	-	0.107	0.01*
RF	0.219	-	0.266
W	0.664	0.129	-

554 **Table 2** Similarity of percentages (SIMPER) analysis of bacterial community dissimilarity (Bray-Curtis) between Reared Restricted (RR), Reared Foraged (RF), and
555 Wild (W) sample group whole microbiota. Given is mean % abundance of sequences for operational taxonomic units across the samples each was observed to
556 occupy and the average dissimilarity between samples ((RR vs. RF) = 58% and (RR vs. W) = 59%, (RF vs. W) = 67%). Percentage contribution is the mean
557 contribution divided by mean dissimilarity across samples. The list of OTUs is not exhaustive so cumulative % value does not sum to 100%. All OTUs listed belong
558 to the common microbiota. Given the length of the ribosomal sequences analyzed, OTU identities should be considered putative.
559

			% Mean abundance					
Class	Family	Taxon name	RR	RF	W	Av. dis.	Cont%	Cuml. %
Betaproteobacteria	Neisseriaceae	*Snodgrassella alvi 99%*	52.1	29.5	22.4	16.16	26.19	26.19
Gammaproteobacteria	Orbaceae	*Gilliamella apicola 99%*	22.3	17.9	30.9	9.50	15.38	41.57
Gammaproteobacteria	Enterobacteriaceae	*Arsenophonus nasoniae 99%*	0.02	6.06	15.8	6.84	11.08	52.65
Flavobacteriia	Flavobacteriaceae	*Flavobacterium* 83%	0.00	9.31	7.76	5.39	8.74	61.39
Bacilli	Lactobacillaceae	*Lactobacillus* 91%	6.72	7.70	1.84	4.31	6.98	68.37
Bacilli	Leuconostocaceae	*Fructobacillus* 100%	0.02	0.29	12.5	4.18	6.78	75.15
Gammaproteobacteria	Enterobacteriaceae	*Yokenella* 98%	7.44	6.04	0.25	4.03	6.52	81.67
Bacilli	Lactobacillaceae	*Lactobacillus kunkeei 100%*	0.17	4.63	3.78	2.86	4.63	86.31
Bacilli	Enterococcaceae	*Vagococcus* 100%	4.47	3.66	0.05	2.50	4.04	90.35
Gammaproteobacteria	Streptococcaceae	*Lactococcus* 98%	0.12	4.92	0.06	1.92	3.11	93.46
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* 100%	2.66	2.27	0.01	1.53	2.48	95.93
Actinobacteria	Bifidobacteriaceae	*Bombiscardovia coagulans 98%*	1.06	1.73	0.87	0.94	1.52	97.46
Bacilli	Enterococcaceae	*Enterococcus* 100%	0.98	1.29	0.04	0.68	1.11	98.56

560

561 **Table 3** Canonical correspondence analyses (CCA) for determination of percent variation in the whole,
562 common, and rare microbiota between the three subject groups by environmental variables significant at the
563 $P < 0.05$ level. CCA biplots are given in Figure S1.

564

	Whole		Common		Rare	
	% variance	*P*	% variance	*P*	% variance	*P*
Foraging	8.44	0.001	8.45	0.001	6.55	0.001
Rearing	7.93	0.002	7.93	0.001	10.74	0.001
Host weight	3.10	0.002	2.76	0.001	10.25	0.001
Undetermined	80.53	-	80.86	-	72.46	-

565

566

567 **Figure S1**

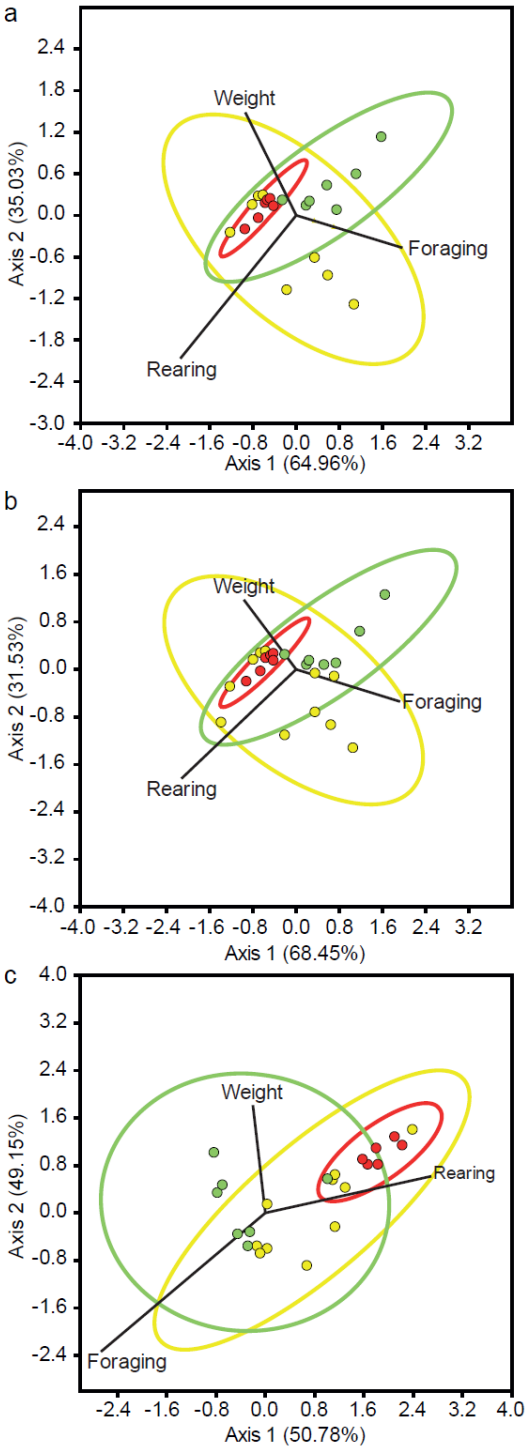


Figure S1 Canonical correspondence bi-plots for (a) whole, (b) common, and (c) rare microbiota. Solid red circles represent microbiota samples from the reared restricted (RR) group, solid yellow circles for the reared foraged (RF) group, and solid green circles for the wild (W) group. In each instance, the 95 % concentration ellipses are given for the RR (red), RF (yellow), and W (green) group microbiota. Bi-plot lines for variables that significantly accounted for variation within the microbiota at the $P < 0.05$ level (see Table 3) show the direction of increase for each variable, and the length of each line indicates the degree of correlation with the ordination axes. CCA field labels: rearing, foraging, and host weight. Percentage of community variation explained by each axis is given in parentheses.

568

569

570 **Table S1** Two-sample *t*-tests comparing mean whole, common, and rare microbiota richness between
571 reared restricted (RR), reared foraged (RF), and wild (W) bee cohorts. Two-sample *t*-test statistic (*t*) and
572 significance (*P*) that richness between two compared groups is significantly different at the $P < 0.05$ level
573 (denoted with asterisks) are given in the lower and upper triangles, respectively.
574

Whole	RR	RF	W
RR	-	0.027*	0.125
RF	2.91	-	0.0001*
W	1.85	11.45	-
Common	RR	RF	W
RR	-	0.054	0.499
RF	2.18	-	0.07
W	0.71	1.96	-
Rare	RR	RF	W
RR	-	0.019*	0.122
RF	3.2	-	0.0001*
W	1.86	12.33	-

575
576
577
578

Table S2 Sample details and barcodes used with their associated samples are given below.

Sample	Origin	Foraged (F) or Restricted (R)	Geography	Total wet weight (g)	Gut wet weight (g)	Barcode Sequence
RR1	Commercially reared	R	n/a	0.175	0.01	GATCTGCGATCC
RR2	Commercially reared	R	n/a	0.194	0.017	AGTCGTGCACAT
RR3	Commercially reared	R	n/a	0.166	0.012	CGAGGGAAAGTC
RR4	Commercially reared	R	n/a	0.233	0.06	CAAATTCGGGAT
RR5	Commercially reared	R	n/a	0.207	0.017	AGATTGACCAAC
RR6	Commercially reared	R	n/a	0.1512	0.016	AGTTTACGAGCTA
RF1	Commercially reared	F	Wallingford	0.251	0.04	CAGCTCATCAGC
RF2	Commercially reared	F	Wallingford	0.308	0.036	CAAACAACAGCT
RF3	Commercially reared	F	Wallingford	0.277	0.042	GCAACACCATCC
RF4	Commercially reared	F	Wallingford	0.176	0.034	GCGATATATCGC
RF5	Commercially reared	F	Wallingford	0.192	0.032	GTATCTGCGCGT
RF6	Commercially reared	F	Wallingford	0.15	0.034	GCATATGCACTG
RF7	Commercially reared	F	Wallingford	0.148	0.028	CAACTCCCGTGA
RF8	Commercially reared	F	Wallingford	0.172	0.01	TTGCGTTAGCAG
RF9	Commercially reared	F	Wallingford	0.143	0.014	TACGAGCCCTAA
RF10	Commercially reared	F	Wallingford	0.286	0.025	ATCACCAGGTGT
W1	Wild	F	Hillesden	0.187	0.017	CGAGCAATCCTA
W2	Wild	F	Hillesden	0.196	0.011	TAATACGGATCG
W3	Wild	F	Hillesden	0.261	0.029	CATTCGTGGCGT
W4	Wild	F	Isle of Wight	0.339	0.03	TCCCTTGTCTCC
W5	Wild	F	Isle of Wight	0.162	0.026	ACGAGACTGATT
W6	Wild	F	Isle of Wight	0.22	0.022	GCTGTACGGATT
W7	Wild	F	Hillesden	0.279	0.03	TGTGAATTCGGA