

Article (refereed) - postprint

Newbold, Lindsay K.; Oliver, Anna E.; Cuthbertson, Leah; Walkington, Sarah E.; Gweon, Hyun S.; Heard, Matthew S.; van der Gast, Christopher J. 2015. **Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota.** *Environmental Microbiology Reports*, 7 (4). 634-641.
[10.1111/1758-2229.12299](https://doi.org/10.1111/1758-2229.12299)

© 2015 Society for Applied Microbiology and John Wiley & Sons Ltd

This version available <http://nora.nerc.ac.uk/511325/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at <http://nora.nerc.ac.uk/policies.html#access>

This document is the author's final manuscript version of the journal article, incorporating any revisions agreed during the peer review process. There may be differences between this and the publisher's version. You are advised to consult the publisher's version if you wish to cite from this article.

The definitive version is available at <http://onlinelibrary.wiley.com/>

Contact CEH NORA team at
noraceh@ceh.ac.uk

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

2

3 Lindsay K. Newbold[†], Anna E. Oliver[†], Leah Cuthbertson, Sarah E. Walkington, Hyun S. Gweon,

4 Matthew S. Heard^{*}, Christopher J. van der Gast^{*}

5

6 *NERC Centre for Ecology & Hydrology, Wallingford, OX10 8BB, UK*

7

8 ^{*} Correspondence: C.J. van der Gast and M.S. Heard, NERC Centre for Ecology & Hydrology,

9 Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, OX10 8BB, UK. E-mail:

10 cjvdg@ceh.ac.uk and mshe@ceh.ac.uk

11

12 [†] Contributed equally to this work

13

14 Running title: Ecological effects on bumblebee gut microbiota

15

16 **Keywords:** *Bombus terrestris* / pollinators / gut microbiome / commonness and rarity / bumblebees /
17 bacterial communities

18

19 Data deposition: The sequence data reported in this paper have been deposited in the European

20 Nucleotide Archive under study accession number ERP007145, and sample accession number

21 ERS557783.

22

23 **Summary**

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

37

38

39 Introduction

40 Insects and other pollinators provide a vital ecosystem service to 87.5% of the world's plant species
41 (Ollerton et al., 2011) and demand for pollination services in crops is high (estimated global value of
42 €153 billion; Gallai et al., 2009). As a consequence, there is an increasing awareness of the
43 ecological and economic importance of such organisms. However, whilst demand for pollination
44 services continues to rapidly increase, there is growing evidence for declines in pollinator populations
45 (Biesmeijer et al., 2006; vanEngelsdorp et al., 2008; Aizen and Harder, 2009; Potts et al., 2010a;
46 Potts et al., 2010b). Declines are likely driven by multiple factors including disease, pesticide use,
47 host plant loss and changes in land management (Cameron et al., 2011; Dicks et al., 2013; Scheper
48 et al., 2014). A link between the reduction of plant pollination, and a drop in pollinator diversity and
49 abundance is also well established (Memmott et al., 2004; Biesmeijer et al., 2006; Albrecht et al.,
50 2012). An increasing human population will only intensify demands on wild and managed pollinator
51 populations to meet future food security needs (Klein et al., 2007; Aizen et al., 2008).

52

53 In temperate systems, eusocial bumblebees (*Bombus* spp.) are important and prolific plant
54 pollinators. Some species are commercially managed to pollinate high value glasshouse and fruit
55 crops (Klein et al., 2007; Leonhardt and Blüthgen, 2012). This practice is increasingly common, with
56 between 30,000-60,000 bumblebee colonies per year being imported into the UK alone (Lye et al.,
57 2011). Ensuring the production of healthy bumblebee colonies will be vital to sustain the growing
58 demand for their services (Pettis et al., 2012). There is therefore interest in how commercially reared
59 bees may differ from wild types in terms of physiology, and how interactions between them may affect
60 fitness (Otterstatter and Thomson, 2008).

61

62 The insect gut is known to harbour a microbial community which is thought to aid host fitness through
63 enhanced nutrition, immunity and colony health (Dillon and Dillon, 2004; Warnecke et al., 2007;
64 Cariveau et al., 2014; Pernice et al., 2014). Recent studies suggest the *Bombus* gut bacterial
65 community is predominately comprised of members from: Orbaceae (Gammaproteobacteria),
66 Lactobacillaceae (Firmicutes), Neisseriaceae (Betaproteobacteria), Acetobacteraceae
67 (Alphaproteobacteria), Bacteroidetes and Actinobacteria (Koch and Schmid-Hempel, 2012; Koch et
68 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

87 **Results and discussion**

88 Bacterial diversity and composition from whole gut samples was assessed using 16S rRNA gene
89 targeted high-throughput sequencing. From 23 bee gut samples, a total of 2,465,708 sequence reads
90 (mean \pm SD per sample, 107204.7 ± 59212.6) were included in the final analysis and 373 distinct
91 operational taxonomic units (OTUs) identified. The average numbers of bacterial sequence reads per
92 sample were similar among the three groups: commercially reared but restricted to colony (RR),
93 $96,484 \pm 55,741$ ($n = 6$); commercially reared but with outside foraging (RF), $100,533 \pm 53,812$ ($n =$
94 10); and wild-caught workers (W), $125,924 \pm 64,867$ ($n = 7$). The number of OTUs we identified is
95 higher than that in studies applying traditional culture independent techniques - ranging from 9 to 146
96 sequenced OTUs (Koch and Schmid-Hempel, 2011b; Martinson et al., 2011). Thus, the increased
97 sampling depth through the application of next generation sequencing (NGS) appears to have
98 captured more of the inherent gut microbial diversity. When compared to other insects guts (e.g up to
99 726 OTUs were identified in the termite hind gut alone, Köhler et al., 2012), an overarching richness
100 of 373 OTUs is relatively low, although comparable to that of the honey bee (Moran et al., 2012),
101 suggesting that the bumblebee gut microbiome does indeed represent a low diversity, specialized
102 community.

103

104 It is expected that a microbial metacommunity would display a positive relationship between
105 frequency and abundance of individual taxa (OTUs) from within its constituent communities (van der
106 Gast et al., 2011). Consistent with this prediction, the abundance of individual bacterial OTUs, across
107 all samples (Figure 1a), was significantly correlated with the number of individual gut sample
108 communities that they occupied. Separating component taxa within a host microbiota into common
109 and rare groupings reveals important aspects of taxa-abundance distributions (van der Gast et al.,
110 2011; van der Gast et al., 2014). Here, we partitioned the OTUs into 'common' (defined as those
111 present in the upper quartile of sample occupancy with $>75\%$ across all samples) and 'rare'
112 groupings. The 28 common OTUs accounted for 97.4% of the total sequence abundance while the
113 rare group comprised the majority of the diversity (345 'rare' OTUs). Similarly, Cariveau et al. (2014)
114 determined that high abundance OTUs represented 98.9% of sequences from *B. bimaculatus* and *B.*
115 *impatiens* gut microbiota samples.

116

117 Mean OTU richness in the whole microbiota was significantly higher within the RF group ($121.5 \pm$
118 10.4 , mean \pm SD) when compared to the other samples (RR, 97.2 ± 18.7 ; and W, 83.0 ± 2.1 ; Figure
119 1b and Table S1). The same significant pattern was reflected in the rare microbiota (RR, 71.2 ± 18.1 ;
120 RF, 96.8 ± 9.8 ; and W, 57.4 ± 2.0), but not in the common microbiota which did not significantly differ
121 between groups (RR, 26.0 ± 1.2 ; RF, 24.7 ± 1.11 ; and W, 25.6 ± 0.8 ; Figure 1b and Table S1). We
122 therefore assert that observed patterns in richness are driven by compositional changes in the rare
123 microbiota. This was confirmed by pair wise comparisons of turnover rates (number of taxa/OTUs
124 eliminated and replaced; Figure 1c), where whole microbiota turnover between groups followed that of
125 the rare microbiota comparisons. No turnover was observed between the common microbiota (Figure
126 1c), however the common microbiota did contribute most to patterns of whole microbiota composition
127 (Figure 1d). Bray-Curtis quantitative index similarity (S_{BC}) revealed the whole microbiota to be highly
128 similar to the corresponding common microbiota (mean $S_{BC} = 0.99 \pm 0.01$, $n = 3$ pair wise
129 comparisons). Conversely, the rare microbiota was highly dissimilar between whole microbiota and
130 corresponding rare microbiota (mean $S_{BC} = 0.04 \pm 0.03$), and were divergent between rare microbiota
131 groups (mean $S_{BC} = 0.23 \pm 0.15$; Figure 1d).

132

133 Analysis of the uniqueness and sample group allocation of OTUs (Figure 2) demonstrated that, in
134 addition to the 28 common OTUs, a further 102 OTUs (taxa) were shared across all treatments.
135 These appear to be an integral part of the wild *B. t. audax* gut microbiota, and therefore likely to be
136 retained across generations. Interestingly, when looking at the allocation of rare OTUs the reared
137 foraging group had the highest number unique of OTUs (75) when compared to the other sample
138 group types (RR = 9, W =13). Further, none of the OTUs detected were shared solely between the
139 RR and W groups, suggesting that although gut microbiota from commercially reared populations are
140 distinct from wild populations, when allowed to forage a shift in microbiota from a commercially reared
141 to wild pattern occurs. As such the RF group would represent a population with microbiota in flux,
142 showing a pattern which shares both commercially reared and wild attributes. If this is the case it
143 would be interesting to consider whether the RF gut microbiota population would fully transition to a
144 wild type and how long such a transition would take. Analysis of similarity (ANOSIM) tests give further
145 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* spp. 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 through 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°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°C for 2 min, individual PCR reactions employed 25 cycles of an initial
237 30 sec, 98°C denaturation step, followed by annealing phase for 30 sec at 53°C , and final extension
238 step lasting 90 secs at 72°C . All reactions employed a final extension step of 5 min at 72°C . 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

241 et al., 2012) and spacer sequences (Table S2). An amplicon library consisting of ~400 bp amplicons
242 spanning the V1-V2 hypervariable regions of the 16S rRNA gene was generated from gel purified
243 pooled products of 4 replicate PCR reactions, per sample. Quantification was performed on an Agilent
244 2200 TapeStation system and an equimolar mix of PCR products was prepared and diluted to 20pM
245 in dH₂O. This library was sequenced using an Ion Torrent Personal Genome Machine (Life
246 Technologies, Paisley, UK) with a 316 chip.

247

248 *Sequence analysis*

249 The Mothur sequencing analysis platform was used to analyse the resulting data (Schloss et al.,
250 2009; Schloss et al., 2011). Sequence quality checks included the removal of failed reads, low-quality
251 ends, tags and primers. Further, sequences were aligned against the Mothur SILVA reference
252 bacterial database and any unaligned sequences that included ambiguous base calls and/or
253 homopolymers longer than 8 bases were also eliminated. Finally, chimeras were identified and
254 discarded through Mothur using the UCHIME algorithm (Edgar et al., 2011). The resultant alignment
255 was used to assemble operational taxonomic unit (OTU) clusters at 96% identity, through distance
256 measures (Schloss and Handelsman, 2005, 2006). Taxonomic identity of these OTUs was assigned
257 using the default settings with the mothur RDP reference database. As an additional measure the
258 identity of reference sequences from key OTUs was corroborated using the NCBI's BLASTN program.
259 OTUs identified in negative controls were removed from further analysis (Salter et al., 2014). The raw
260 sequence data reported in this study have been deposited in the European Nucleotide Archive under
261 study accession number ERP007145 and sample accession number ERS557783. The relevant
262 barcode information for each sample is shown in Table S2.

263

264 *Statistical analysis*

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

271 differences in sample size (Gihring et al., 2012). Therefore, S^* was calculated with a uniform re-
272 sample size (to match the smallest sequence size in each microbiota group [whole, common, and
273 rare]) following 1000 iterations in each instance and performed in R version 3.1.1 (Oksanen et al.,
274 2013; The R Development Core Team, 2013)

275

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

293

294

295 **Acknowledgments**

296 This work was supported by the NERC Centre for Ecology & Hydrology (CEH). We thank Sarah
297 Hulmes, Lucy Hulmes, Jo Savage, and Claire Carvell (all CEH) for field sample collections.

298 **References**

- 299 Aizen, M.A., and Harder, L.D. (2009) The Global Stock of Domesticated Honey Bees Is Growing
300 Slower Than Agricultural Demand for Pollination. *Curr Biol* **19**: 915-918.
301
- 302 Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., and Klein, A.M. (2008) Long-Term Global Trends in
303 Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator
304 Dependency. *Curr Biol* **18**: 1572-1575.
305
- 306 Albrecht, M., Schmid, B., Hautier, Y., and Müller, C.B. (2012) Diverse pollinator communities enhance
307 plant reproductive success. *Proc R Soc B* **279**: 4845-4852
308
- 309 Babendreier, D., Joller, D., Romeis, J., Bigler, F., and Widmer, F. (2007) Bacterial community
310 structures in honeybee intestines and their response to two insecticidal proteins. *FEMS Microbiol Ecol*
311 **59**: 600-610.
312
- 313 Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T. et al. (2006)
314 Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science*
315 **313**: 351-354.
316
- 317 Brown, J.H., and Kodric-Brown, A. (1977) Turnover rates in insular biogeography: Effect of
318 immigration and extinction. *Ecology* **58**: 445-449.
319
- 320 Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., and Griswold, T.L.
321 (2011) Patterns of widespread decline in North American bumble bees. *P Natl Acad Sci USA* **108**:
322 662-667.
323
- 324 Cariveau, D.P., Elijah Powell, J., Koch, H., Winfree, R., and Moran, N.A. (2014) Variation in gut
325 microbial communities and its association with pathogen infection in wild bumble bees (*Bombus*).
326 *ISME J* **8**: 2369-2379.
327
- 328 Carvell, C., Jordan, W.C., Bourke, A.F.G., Pickles, R., Redhead, J.W., and Heard, M.S. (2012)
329 Molecular and spatial analyses reveal links between colony-specific foraging distance and landscape-
330 level resource availability in two bumblebee species. *Oikos* **121**: 734-742.
331
- 332 Darby, A.C., Choi, J.H., Wilkes, T., Hughes, M.A., Werren, J.H., Hurst, G.D., and Colbourne, J.K.
333 (2010) Characteristics of the genome of *Arsenophonus nasoniae*, son-killer bacterium of the wasp
334 *Nasonia*. *Insect Mol Biol* **19 Suppl 1**: 75-89.
335
- 336 Dicks, L.V., Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C. et al. (2013) Identifying
337 key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative
338 cross-sectoral exercise. *Insect Conserv Diver* **6**: 435-446.
339
- 340 Dillon, R.J., and Dillon, V.M. (2004) The Gut Bacteria of Insects: Nonpathogenic Interactions. *Annual*
341 *Review of Entomology* **49**: 71-92.
342
- 343 Duron, O. (2014) *Arsenophonus* insect symbionts are commonly infected with APSE, a bacteriophage
344 involved in protective symbiosis. *FEMS Microbiol Ecol* **90**: 184-194
345
- 346 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves
347 sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194-2200.
348
- 349 Endo, A., and Salminen, S. (2013) Honeybees and beehives are rich sources for fructophilic lactic
350 acid bacteria. *Syst Appl Microbiol* **36**: 444-448.
351
- 352 Engel, P., Stepanauskas, R., and Moran, N.A. (2014) Hidden Diversity in Honey Bee Gut Symbionts
353 Detected by Single-Cell Genomics. *PLoS Genet* **10**: e1004596.
354
- 355 Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B.E. (2009) Economic valuation of the vulnerability
356 of world agriculture confronted with pollinator decline. *Ecol Econ* **68**: 810-821.

357
358 Gihring, T.M., Green, S.J., and Schadt, C.W. (2012) Massively parallel rRNA gene sequencing
359 exacerbates the potential for biased community diversity comparisons due to variable library sizes.
360 *Environ Microbiol* **14**: 285-290.

361
362 Hazard, C., Gosling, P., van der Gast, C.J., Mitchell, D.T., Doohan, F.M., and Bending, G.D. (2013)
363 The role of local environment and geographical distance in determining community composition of
364 arbuscular mycorrhizal fungi at the landscape scale. *ISME J* **7**: 498-508.

365
366 Killer, J., Votavová, A., Valterová, I., Vlková, E., Rada, V., and Hroncová, Z. (2014a) *Lactobacillus*
367 *bombi* sp. nov., from the digestive tract of laboratory-reared bumblebee queens (*Bombus terrestris*).
368 *Int J Syst Evol Micro* **64**: 2611-2617.

369
370 Killer, J., Kopečný, J., Mrázek, J., Havlík, J., Koppová, I., Benada, O. et al. (2010) *Bombiscardovia*
371 *coagulans* gen. nov., sp. nov., a new member of the family Bifidobacteriaceae isolated from the
372 digestive tract of bumblebees. *Syst Appl Microbiol* **33**: 359-366.

373
374 Killer, J., Švec, P., Sedláček, I., Černošková, J., Benada, O., Hroncová, Z. et al. (2014b)
375 *Vagococcus entomophilus* sp. nov., from the digestive tract of a wasp (*Vespula vulgaris*). *Int J Syst*
376 *Evol Micro* **64**: 731-737.

377
378 Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and
379 Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B*
380 **274**: 303-313.

381
382 Koch, H., and Schmid-Hempel, P. (2011a) Socially transmitted gut microbiota protect bumble bees
383 against an intestinal parasite. *Proc Natl Acad Sci USA* **108**: 19288-19292.

384
385 Koch, H., and Schmid-Hempel, P. (2011b) Bacterial communities in central European bumblebees:
386 low diversity and high specificity. *Microbial Ecol* **62**: 121-133.

387
388 Koch, H., and Schmid-Hempel, P. (2012) Gut microbiota instead of host genotype drive the specificity
389 in the interaction of a natural host-parasite system. *Ecol Lett* **15**: 1095-1103.

390
391 Koch, H., Cisarovsky, G., and Schmid-Hempel, P. (2012) Ecological effects on gut bacterial
392 communities in wild bumblebee colonies. *J Anim Ecol* **81**: 1202-1210.

393
394 Koch, H., Abrol, D.P., Li, J., and Schmid-Hempel, P. (2013) Diversity and evolutionary patterns of
395 bacterial gut associates of corbiculate bees. *Mol Ecol* **22**: 2028-2044.

396
397 Köhler, T., Dietrich, C., Scheffrahn, R.H., and Brune, A. (2012) High-Resolution Analysis of Gut
398 Environment and Bacterial Microbiota Reveals Functional Compartmentation of the Gut in Wood-
399 Feeding Higher Termites (*Nasutitermes* spp.). *Appl Environ Microb* **78**: 4691-4701.

400
401 Kosako, Y., Sakazaki, R., and Yoshizaki, E. (1984) *Yokenella regensburgei* gen. nov., sp. nov.: a new
402 genus and species in the family Enterobacteriaceae. *Jpn J Med Sci Biol* **37**: 117-124.

403
404 Kwong, W.K., and Moran, N.A. (2013) Cultivation and characterization of the gut symbionts of honey
405 bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family
406 *Neisseriaceae* of the *Betaproteobacteria*, and *Gilliamella apicola* gen. nov., sp. nov., a member of
407 *Orbaceae* fam. nov., *Orbales* ord. nov., a sister taxon to the order 'Enterobacteriales' of the
408 *Gammaproteobacteria*. *Int J Syst Evol Microbiol* **63**: 2008-2018.

409
410 Kwong, W.K., Mancenido, A.L., and Moran, N.A. (2014a) Genome Sequences of *Lactobacillus* sp.
411 Strains wkB8 and wkB10, Members of the Firm-5 Clade, from Honey Bee Guts. *Genome Announc* **2**.

412
413 Kwong, W.K., Engel, P., Koch, H., and Moran, N.A. (2014b) Genomics and host specialization of
414 honey bee and bumble bee gut symbionts. *Proc Natl Acad Sci USA* **111**: 11509-11514.

415

416 Leonhardt, S., and Blüthgen, N. (2012) The same, but different: pollen foraging in honeybee and
417 bumblebee colonies. *Apidologie* **43**: 449-464.
418

419 Lye, G.C., Jennings, S.N., Osborne, J.L., and Goulson, D. (2011) Impacts of the use of Nonnative
420 Commercial Bumble Bees for Pollinator Supplementation in Raspberry. *J Econ Entomol* **104**: 107-
421 114.
422

423 Martinson, V.G., Danforth, B.N., Minckley, R.L., Rueppell, O., Tingek, S., and Moran, N.A. (2011) A
424 simple and distinctive microbiota associated with honey bees and bumble bees. *Mol Ecol* **20**: 619-
425 628.
426

427 Memmott, J., Waser, N.M., and Price, M.V. (2004) Tolerance of pollination networks to species
428 extinctions. *Proc R Soc B* **271**: 2605-2611.
429

430 Moran, N.A., Hansen, A.K., Powell, J.E., and Sabree, Z.L. (2012) Distinctive Gut Microbiota of Honey
431 Bees Assessed Using Deep Sampling from Individual Worker Bees. *PLoS ONE* **7**: e36393.
432

433 Novakova, E., Hypsa, V., and Moran, N. (2009) *Arsenophonus*, an emerging clade of intracellular
434 symbionts with a broad host distribution. *BMC Microbiol* **9**: 143.
435

436 Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. et al. (2013)
437 *Package vegan: community ecology package. R package version 2.0-7*. Vienna, Austria.: R
438 Foundation for Statistical Computing.
439

440 Ollerton, J., Winfree, R., and Tarrant, S. (2011) How many flowering plants are pollinated by animals?
441 *Oikos* **120**: 321-326.
442

443 Otterstatter, M.C., and Thomson, J.D. (2008) Does Pathogen Spillover from Commercially Reared
444 Bumble Bees Threaten Wild Pollinators? *PLoS ONE* **3**: e2771.
445

446 Pettis, J.S., van Engelsdorp, D., Johnson, J., and Dively, G. (2012) Pesticide exposure in honey bees
447 results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften* **99**: 153-158.

448 Pernice, M., Simpson, S.J., and Ponton, F. (2014) Towards an integrated understanding of gut
449 microbiota using insects as model systems. *Journal of Insect Physiology* **69**: 12-18.
450

451 Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. (2010a)
452 Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* **25**: 345-353.
453

454 Potts, S.G., Roberts, S.P.M., Dean, R., Marris, G., Brown, M.A., Jones, R. et al. (2010b) Declines of
455 managed honey bees and beekeepers in Europe. *J Apicult Res* **49**: 15-22.
456

457 Powell, J.E., Martinson, V.G., Urban-Mead, K., and Moran, N.A. (2014) Routes of acquisition of the
458 gut microbiota of *Apis mellifera*. *Appl Environ Microbiol*. Accepted manuscript posted online sept
459 2014
460

461 Rogers, G.B., Cuthbertson, L., Hoffman, L.R., Wing, P.A.C., Pope, C., Hooftman, D.A.P. et al. (2013)
462 Reducing bias in bacterial community analysis of lower respiratory infections. *ISME J* **7**: 697-706.
463

464 Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F. et al. (2014) Reagent
465 and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol*
466 **12**: 87
467

468 Scheper, J., Reemer, M., van Kats, R., Ozinga, W.A., van der Linden, G.T.J., Schaminée, J.H.J. et al.
469 (2014) Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in
470 The Netherlands. *Proc Natl Acad Sci USA* **111**: 17552-17557.
471

472 Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a Computer Program for Defining
473 Operational Taxonomic Units and Estimating Species Richness. *Appl Environ Microbiol* **71**: 1501-
474 1506.

475
476 Schloss, P.D., and Handelsman, J. (2006) Introducing SONS, a Tool for Operational Taxonomic Unit-
477 Based Comparisons of Microbial Community Memberships and Structures. *Appl Environ Microbiol* **72**:
478 6773-6779.
479
480 Schloss, P.D., Gevers, D., and Westcott, S.L. (2011) Reducing the effects of PCR amplification and
481 sequencing artifacts on 16S rRNA-based studies. *PLoS ONE* **6**: e27310.
482
483 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B. et al. (2009)
484 Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for
485 Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75**: 7537-7541.
486
487 The R Development Core Team (2013) *R: a language and environment for statistical computing*.
488 Vienna, Austria: R Foundation for Statistical Computing.
489
490 van der Gast, C.J., Walker, A.W., Stressmann, F.A., Rogers, G.B., Scott, P., Daniels, T.W. et al.
491 (2011) Partitioning core and satellite taxa from within cystic fibrosis lung bacterial communities. *ISME*
492 *J* **5**: 780-791.
493
494 van der Gast, C.J., Cuthbertson, L., Rogers, G.B., Pope, C., Marsh, R.L., Redding, G.J. et al. (2014)
495 Three clinically distinct chronic pediatric airway infections share a common core microbiota. *Ann Am*
496 *Thorac Soc* **11**: 1039-1048.
497
498 vanEngelsdorp, D., Hayes, J., Jr., Underwood, R.M., and Pettis, J. (2008) A Survey of Honey Bee
499 Colony Losses in the U.S., Fall 2007 to Spring 2008. *PLoS ONE* **3**: e4071.
500
501 Warnecke, F., Luginbuhl, P., Ivanova, N., Ghassemian, M., Richardson, T.H., Stege, J.T. et al. (2007)
502 Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*
503 **450**: 560-565.
504
505 Whiteley, A.S., Jenkins, S., Waite, I., Kresoje, N., Payne, H., Mullan, B. et al. (2012) Microbial 16S
506 rRNA Ion Tag and community metagenome sequencing using the Ion Torrent (PGM) Platform. *J*
507 *Microbiol Method* **91**: 80-88.
508
509 Wilkes, T.E., Duron, O., Darby, A.C., Hypa, V., Nováková, E., and Hurst, G.D.D. (2011) The Genus
510 *Arsenophonus*. In *Manipulative Tenants: Bacteria Associated with Arthropods*, Zchori-Fein, E., and
511 Bourtzis, K. (eds). Danvers, M A: CRC Press, pp. 225-244.
512
513
514

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

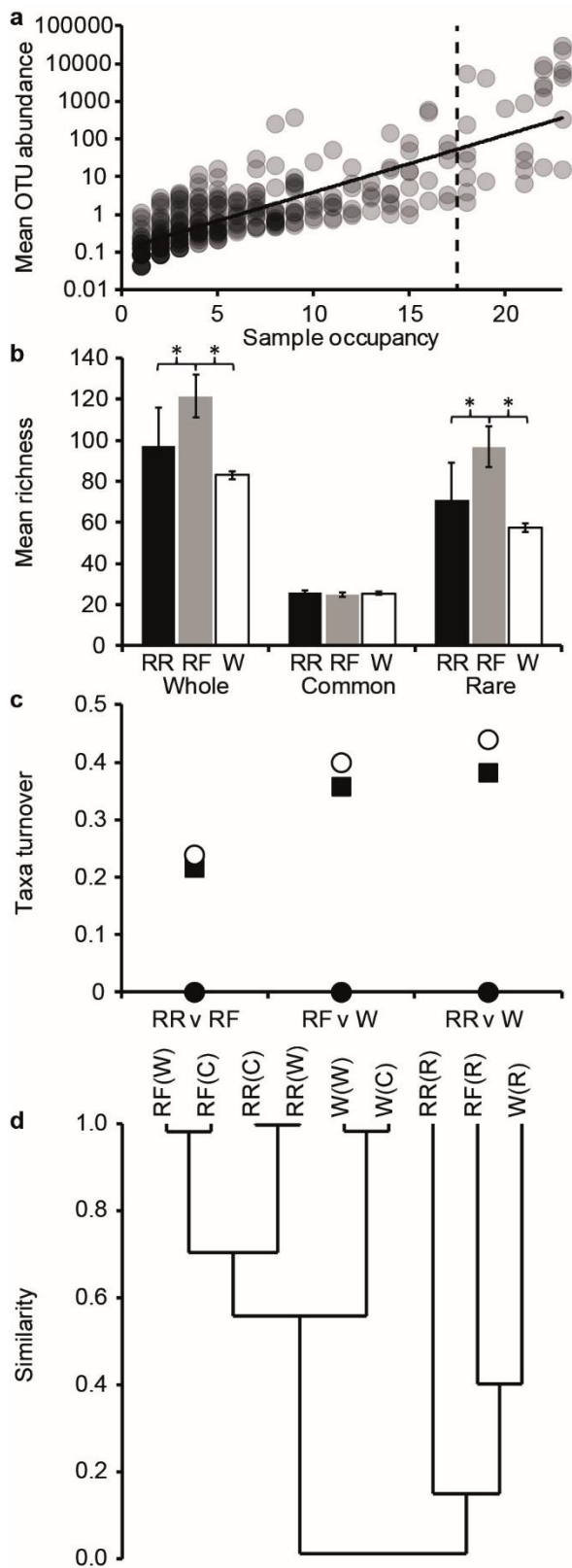
530 **Figure 2** Unique and shared OTUs between groups. Values given within circles represent, unique
531 OTUs to the reared restricted (RR) group, reared foraged (RF), and wild (W) groups. Values given in
532 overlapping regions correspond to the number of OTUs shared between two given groups. Central
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

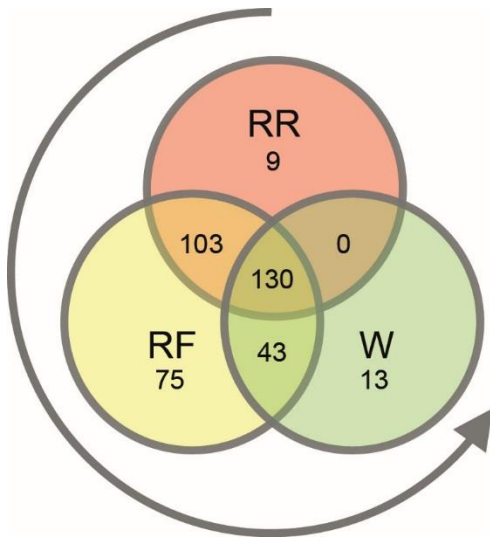
539 **Figure 1**



540

541

542 **Figure 2**



543

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

549
 550

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	-

551
 552
 553

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

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



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

579 **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	CAAATTCGGAT
RR5	Commercially reared	R	n/a	0.207	0.017	AGATTGACCAAC AGTTTACGAGCT
RR6	Commercially reared	R	n/a	0.1512	0.016	A
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	TCCCTTGCTCC
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

580

581

582