

Supplementary Materials for

The gut microbiome defines social group membership in honey bee colonies

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/42/eabd3431/DC1)

Data files S1 to S4

Table S1. Individual microbial taxa differ in read counts between foragers from three different honey bee colonies. Only taxa that significantly differ between groups are included. Numbers represent mean read count \pm standard error across ten biological replicates. All p-values are from nonparametric Kruskal Wallis ANOVA (KW). Letters denote statistically significant age groups across individual compounds via Dunn's Test with FDR adjustment (KW post-hoc) ($p < 0.05$).

ASV	Likely microbe	Relationship to Honey bee	Colony 1	Colony 2	Colony 3	KW p-value
9f6cc04b4b4b011455a72b5a60460c23	<i>Fructobacillus</i> sp.	NA	0 \pm 0 (A)	22.2 \pm 11.9 (B)	0 \pm 0 (A)	0.001
84ec1399e8ee3618cc0686f28b89dbcb	<i>Erwinia</i> sp.	Opportunistic, plant pathogen	2.3 \pm 2.3 (A)	13.9 \pm 6.1 (B)	0 \pm 0 (A)	0.044
3d6c6053fcc0b58354ac9db e2d2f699e	<i>Acinetobacter</i> sp.	NA	0.6 \pm 0.6 (A)	229.9 \pm 98.4 (B)	18.7 \pm 18.7 (A)	0.001
35a3d8346211c36fb8c761f16cf80a78	<i>Lactobacillus kunkeei</i>	Symbiont	54.7 \pm 14.6 (A)	1 \pm 1 (B)	16.4 \pm 13 (B)	0.001
74b24ccc7660cd841e826ef e9a2ab1b0	<i>Bombella apis</i>	Symbiont	51.5 \pm 24.5 (A)	0 \pm 0 (B)	0.3 \pm 0.3 (B)	0.002
bc33fe4f622661dee912ccfb ead7c189	<i>Lactobacillus Firm-5</i>	Symbiont	55.2 \pm 33 (A)	21.8 \pm 6.5 (A)	117.2 \pm 24.9 (B)	0.023
49ba1666cb48aa5757c0f882dc2362	<i>Lonsdalea</i> sp.	Opportunistic, plant pathogen	0 \pm 0 (A)	198.5 \pm 91.6 (B)	619.7 \pm 160.3 (B)	0.001
4c93c2752e221ba62f741be f52a062ae	<i>Bacillus</i> sp.	NA	0 \pm 0 (A)	4.1 \pm 2.6 (B)	0 \pm 0 (A)	0.04
1bfdde56f0549c7953359140bb8c64b1	<i>Serratia marcescens</i>	Opportunistic, pathogen	0 \pm 0 (A)	12.1 \pm 7.2 (B)	0 \pm 0 (A)	0.04
add077c21c9d0c2e6d5a9c3073a9f2ff	<i>Bartonella apis</i>	Symbiont	216.5 \pm 70.9 (A)	20.6 \pm 12.9 (B)	37.1 \pm 22.3 (B)	0.002
d945fa3999147e0e192657a812a81864	<i>Erwinia</i> sp.	Opportunistic, plant pathogen	0 \pm 0 (A)	0 \pm 0 (A)	9.7 \pm 5 (B)	0.012
60f0a102e1b3118286adc774264b5b7d	<i>Leuconostoc</i> sp.	NA	4.5 \pm 2.5 (A)	0 \pm 0 (B)	0 \pm 0 (B)	0.04
e0f189a223266cebe35ecce782442771	<i>Lactobacillus Firm-4</i>	Symbiont	156.5 \pm 81 (A)	48.8 \pm 16.4 (A)	294.2 \pm 86.8 (B)	0.03
cbc8ca79561859eef1323f7b78e61b0c	<i>Bombella</i> sp.	Symbiont	302.9 \pm 144.4 (A)	190.6 \pm 139.1 (AB)	0 \pm 0 (B)	0.05
677929eac52be081cdae08a0b9c70eb2	<i>Klebsiella</i> sp.	NA	22.2 \pm 22.2 (A)	57.9 \pm 30.4 (B)	0 \pm 0 (A)	0.02
53487488977aaff4786546cbeeb53194	Bacteroidetes	NA	135.6 \pm 70.1 (A)	0 \pm 0 (B)	0 \pm 0 (B)	0.001
8fc75f0f847f0a7817148a589a678a9b	Halomonadaceae (<i>Zymobacter</i> sp.)	Environmental, found in beebread	0 \pm 0 (A)	93.6 \pm 40.9 (B)	91.1 \pm 73.2 (B)	0.001
1357277b4f47cd8732a2707eca12fcdff	<i>Leuconostoc mesenteroides</i>	NA	0 \pm 0 (A)	159.3 \pm 70.3 (B)	0 \pm 0 (A)	0.001
e10a68e9c495ac6e874cd1fc707f2ed0	Acetobacteraceae (<i>Gluconobacter</i> sp.)	Possible symbiont	0 \pm 0 (A)	0.2 \pm 0.2 (A)	6.9 \pm 3.8 (B)	0.038
255710e5f82efe52eed0fba9c739b4b	<i>Fructobacillus</i> sp.	NA	0.3 \pm 0.3 (A)	6.2 \pm 2.5 (B)	0.7 \pm 0.7 (A)	0.039
59cc3eb4a94a68b8e3f5a8a2559211f8	<i>Bartonella apis</i>	Symbiont	1136.6 \pm 478.5 (A)	368.5 \pm 193.3 (A)	375.8 \pm 374.9 (B)	0.011
c40e998dab561c150123e6f1d70eb023	<i>Fructobacillus</i> sp.	NA	0 \pm 0 (A)	41.8 \pm 18.7 (B)	0 \pm 0 (A)	0.003
5228abf2f3f8c49b39b8b28a2d51ca69	<i>Dickeya</i> sp.	Opportunistic, plant pathogen	0 \pm 0 (A)	1.1 \pm 1.1 (A)	20.9 \pm 13.5 (B)	0.014

1ebd3e8b2c26aad6622cd55 14b794a67	Erwinia sp.	Opportunistic, plant pathogen	0 ± 0 (A)	2.2 ± 2.2 (A)	24 ± 11.5 (B)	0.004
a310dd8d6ac8e59532216a8 7b485e069	Lactobacillus Firm-5	Symbiont	114.4 ± 53.1 (A)	65.2 ± 16.1 (A)	195.7 ± 26.4 (B)	0.004
cc1f555fcb571340d624aafc e016fbfa	Lactococcus sp.	Symbiont	0 ± 0 (A)	49.1 ± 30.9 (B)	0 ± 0 (A)	0.001

Table S2. Individual microbial taxa differ in read counts between cross-fostered foragers. Only taxa that significantly differ between groups are included. Numbers represent mean read count \pm standard error across ten biological replicates. All p-values are from nonparametric Kruskal Wallis ANOVA (KW). Letters denote statistically significant age groups across individual compounds via Dunn's Test with FDR adjustment (KW post-hoc) ($p < 0.05$).

ASV	Likely microbe	Relationship to Honey bee	Colony 1 raised in Colony 1	Colony 2 raised in Colony 1	Colony 1 raised in Colony 2	Colony 2 raised in Colony 2	KW p-value
11c8b11583b08e1c7c27279aa9419a7a	<i>Frischella perrara</i>	Symbiont	0 \pm 0 (A)	42.4 \pm 28.2 (B)	0 \pm 0 (A)	0 \pm 0 (A)	0.031
202f673d1c8c89a33736db2940b04f04	<i>Snodgrassella alvi</i>	Symbiont	23.6 \pm 8.5 (A)	19.3 \pm 5.6 (A)	2.1 \pm 2.1 (B)	2.9 \pm 2.1 (B)	0.020
35a3d8346211c36fb8c761f16cf80a78	<i>Lactobacillus kunkeei</i>	Symbiont	4.6 \pm 2 (A)	6.6 \pm 2 (A)	0 \pm 0 (B)	0.2 \pm 0.2 (B)	0.004
49ba1666cb48aa5757c0f882dc2362	<i>Lonsdalea</i> sp.	Opportunistic, plant pathogen	0.1 \pm 0.1 (A)	0.4 \pm 0.4 (A)	155.6 \pm 73.6 (B)	28.8 \pm 13.3 (A)	0.002
53487488977aff4786546cbeeb53194	<i>Apibacter</i> sp.	Symbiont	30.1 \pm 15 (A)	16.1 \pm 9.3 (A)	0 \pm 0 (B)	0 \pm 0 (B)	0.008
59cc3eb4a94a68b8e3f5a8a2559211f8	<i>Bartonella apis</i>	Symbiont	179.7 \pm 73.8 (A)	116 \pm 51.2 (A)	2.4 \pm 1.4 (B)	13.5 \pm 10 (B)	0.007
6d9c4b079d57e3814cf9b4ee45e6f4c9	<i>Apibacter</i> sp.	Symbiont	24.7 \pm 16	19 \pm 9.6	0 \pm 0	0 \pm 0	0.044
7931a772085e7c872f91e125c9d82ba9	Acetobacteraceae (<i>Gluconobacter</i> sp.)	Possible symbiont	14 \pm 13.7 (A)	0 \pm 0 (B)	0 \pm 0 (B)	0 \pm 0 (B)	0.047
8a26a9bd241e9a9d74a91777512498d6	<i>Frischella perrara</i>	Symbiont	49.1 \pm 25.3 (A)	79.4 \pm 42.2 (A)	22.5 \pm 16.2 (A)	247.1 \pm 39 (B)	0.002
8fc75f0f847f0a7817148a589a678a9b	Halomonadaceae (<i>Zymobacter</i> sp.)	Environmental, found in beebread	0 \pm 0 (A)	0 \pm 0 (A)	11.5 \pm 7.1 (B)	0 \pm 0 (A)	0.016
a175afda351eb929ddb684ce81f739f3	<i>Lonsdalea</i> sp.	Opportunistic, plant pathogen	0 \pm 0 (A)	0 \pm 0 (A)	146.4 \pm 73.9 (B)	34.6 \pm 11.3 (B)	0.002
b4cc73c05f74abaa779ff9ed5f745c21	<i>Bartonella apis</i>	Symbiont	18.3 \pm 13.2 (A)	0 \pm 0 (B)	0 \pm 0 (B)	0 \pm 0 (B)	0.047
caa792085f9da84dffef5d8a536329d5	<i>Frischella perrara</i>	Symbiont	58.9 \pm 23 (A)	84.7 \pm 41.3 (A)	21.5 \pm 14.4 (A)	252.1 \pm 38.5 (B)	0.003
ff06926c491e77646be2105dfc0c2635	<i>Dickeya</i> sp.	Opportunistic, plant pathogen	0 \pm 0 (A)	0 \pm 0 (A)	25.8 \pm 14.7 (B)	0 \pm 0 (A)	0.003

Table S3. Individual microbial taxa differ in read counts between sister bees inoculated with heat killed- or live- inoculum. Only taxa that significantly differ between groups are included. Numbers represent mean read count \pm standard error across ten biological replicates. All p-values are from nonparametric Mann-Whitney U test (MWU).

ASV	Likely microbe	Relationship to Honey bee	Heat killed	Live	MWU p-value
35a3d8346211c36fb8c761f16cf80a78	<i>Lactobacillus kunkeei</i>	Symbiont	290.4 \pm 27.4	93.3 \pm 8.8	0.001
41bdbe7bfb2c3adb74951a708a05174	<i>Klebsiella</i> sp.	NA	0 \pm 0	5.8 \pm 3.1	0.04
45d402e9427dc221dc55fa21dcbd25c9	<i>Pantoea</i> sp.	NA	0 \pm 0	77.6 \pm 19.8	0.004
59cc3eb4a94a68b8c3f5a8a2559211f8	<i>Bartonella apis</i>	Symbiont	217.5 \pm 61.8	468.9 \pm 66.8	0.017
5dd1c7e6606cce8fae641fab62e3ff5b	<i>Spiroplasma</i> sp.	NA	0 \pm 0	9.7 \pm 4.5	0.01
668c3a8ab32d4d8fc8d35aed81618ca4	Halomonadaceae (<i>Zymobacter</i> sp.)	Environmental, found in beebread	0 \pm 0	1.6 \pm 1.1	0.02
68f99c11da883663f9f535d4b6c3f942	<i>Pantoea</i> sp.	NA	0 \pm 0	75 \pm 18.6	0.001
add077c21c9d0c2e6d5a9c3073a9f2ff	<i>Bartonella apis</i>	Symbiont	0 \pm 0	2.8 \pm 1.4	0.04
c8bb41a915e4bc6b63d2e697332979c9	Acetobacteraceae (<i>Gluconobacter</i> sp.)	Possible symbiont	0 \pm 0	16.5 \pm 7.1	0.001
caa792085f9da84dffef5d8a536329d5	<i>Lonsdalea quercina</i>	Opportunistic, plant pathogen	74.4 \pm 28	23 \pm 10.7	0.025
d74973c26f94dd14c74b70f136599b53	<i>Serratia</i> sp.	Opportunistic, plant pathogen	0 \pm 0	5.8 \pm 2.7	0.02
d945fa3999147e0e192657a812a81864	<i>Serratia</i> sp.	Opportunistic, plant pathogen	0 \pm 0	3.5 \pm 2	0.04
e4d48ac9b1335045533830ed6d8617ac	<i>Bombella apis</i>	Symbiont	37.5 \pm 13.3	0 \pm 0	0.001

Table S4. Individual microbial taxa differ in read counts between sister bees raised with foragers from two different honey bee colonies. Only taxa that significantly differ between groups are included. Numbers represent mean read count \pm standard error across ten biological replicates. All p-values are from nonparametric Mann-Whitney U test (MWU).

ASV	Likely microbe	Relationship to Honey bee	Raised with colony 1 bees	Raised with colony 2 bees	MWU p-value
35a3d8346211c36fb8c761f16cf80a78	<i>Lactobacillus kunkeei</i>	Symbiont	0.2 \pm 0.2	19.1 \pm 12.9	0.026
3e465a6bd730f0113d854c9535cc91f9	<i>Lactobacillus mellis</i>	Symbiont	19.8 \pm 3.6	0 \pm 0	0.001
59cc3eb4a94a68b8e3f5a8a2559211f8	<i>Bartonella apis</i>	Symbiont	489.9 \pm 90.9	11.7 \pm 11.7	0.001
74b24ccc7660cd841e826efe9a2ab1b0	<i>Bombella apis</i>	Symbiont	0 \pm 0	6.2 \pm 2.9	0.035
7fc460b386431099c45986947f6d800f	<i>Bifidobacterium asteroides</i>	Symbiont	48.8 \pm 7.6	0.7 \pm 0.7	0.001
829b1795d54b70a746e61cfd52e45ea9	<i>Snodgrassella alvi</i>	Symbiont	143.1 \pm 43.8	0 \pm 0	0.001
a310dd8d6ac8e59532216a87b485e069	<i>Lactobacillus apis</i>	Symbiont	60.1 \pm 22	2.6 \pm 2.6	0.001
b7420af7805b65881f319c0276233813	<i>Lactobacillus kullabergensis</i>	Symbiont	35.1 \pm 11.5	5.8 \pm 5.8	0.001
be33fe4f622661dee912ccfbeat7c189	<i>Lactobacillus melliventris</i>	Symbiont	32.8 \pm 8.5	22.8 \pm 19.3	0.009
e4d48ac9b1335045533830ed6d8617ac	<i>Bombella apis</i>	Symbiont	0.4 \pm 0.2	390.1 \pm 148.1	0.001

Table S5. *G. apicola* and *L. quercina* were common in the guts of all forager bees used throughout this study. Proportion of 16S rRNA sequencing reads that corresponded to *G. apicola* and *L. quercina*. Numbers represent mean proportion of reads per sample \pm standard error.

Experiment	Corresponding figure	Treatment	Collection Location	Year collected	<i>G. apicola</i>	<i>L. quercina</i>
Foragers from 3 colonies	Fig. 1A-C	Colony 1	Residence in St. Louis, MO	2017	0.394 \pm 0.047	0 \pm 0
		Colony 2	Field station in Eureka, MO	2017	0.423 \pm 0.052	0.018 \pm 0.008
		Colony 3	Field station in Eureka, MO	2017	0.326 \pm 0.052	0.053 \pm 0.014
Cross-fostered foragers	Fig. 1D	Colony 1 raised in Colony 1	Residence in St. Louis, MO	2018	0.307 \pm 0.050	0.001 \pm 0.001
		Colony 2 raised in Colony 1	Residence in St. Louis, MO	2018	0.342 \pm 0.052	0.001 \pm 0.001
		Colony 1 raised in Colony 2	Field station in Eureka, MO	2018	0.319 \pm 0.059	0.168 \pm 0.082
		Colony 2 raised in Colony 2	Field station in Eureka, MO	2018	0.342 \pm 0.052	0.035 \pm 0.012

Table S6. Specific microbial taxa that differed in read counts between sister bees inoculated with either *G. apicola* or *L. quercina*. Only taxa that significantly differed between groups are included. Numbers represent mean read count \pm standard error across ten biological replicates. All p-values are from nonparametric Mann-Whitney U test (MWU).

ASV	Likely microbe	Relationship to Honey bee	Inoculated with <i>G. apicola</i>	Inoculated with <i>L. quercina</i>	MWU p-value
d945fa3999147e0e192657a812a81864	<i>Gibbsiella</i> sp.	Opportunistic	134.2 \pm 42.9	1.4 \pm 1	0.001
3e465a6bd730f0113d854c9535cc91f9	<i>Lactobacillus</i> sp.	Symbiont	13.1 \pm 4.5	3 \pm 3	0.033
1b158b8b2922d4fcad5d9cea607cbb7d	<i>Escherichia coli</i>	Opportunistic	9.3 \pm 9.3	25.7 \pm 6.7	0.006
1bfdde56f0549c7953359140bb8c64b1	<i>Lonsdalea quercina</i>	Opportunistic, plant pathogen	0 \pm 0	106.1 \pm 32.3	0.001
1c2d9bebac9fdb263c581d255419c9a5	<i>Gilliamella apicola</i>	Symbiont	130.5 \pm 56.3	29.9 \pm 29.9	0.009

Table S7. Microbial taxa present in gut inoculum. Numbers represent read count in freshly dissected guts (fresh) and inoculum in 25% sugar water after 24 hours of incubation (inoculum).

ASV	Likely microbe	Relationship to honey bee	Fresh	Inoculum 1	Inoculum 2	Inoculum 3
0c824386f57c0616a1aa709e2c459462	<i>Lactobacillus mellifer</i>	Symbiont	3	11	5	8
1c2d9bebac9fdb263c581d255419c9a5	<i>Gilliamella apicola</i>	Symbiont	304	125	158	176
233b3fd5526a90bb3849012302ef242e	<i>Snodgrassella alvi</i>	Symbiont	0	0	7	0
37882b6bb8c8dbbfbff3072efb27e6a400	<i>Snodgrassella alvi</i>	Symbiont	331	303	43	44
3e465a6bd730f0113d854c9535cc91f9	<i>Lactobacillus mellis</i>	Symbiont	0	0	14	7
43ff6815fcebfb8aeba2478e36cd54ed	<i>Acetobacteraceae</i>	Symbiont	0	17	0	0
4f692b53bc7ee9a4e46d65412d545dc5	<i>Acetobacteraceae</i>	Symbiont	0	0	12	13
59cc3eb4a94a68b8e3f5a8a2559211f8	<i>Bartonella apis</i>	Symbiont	234	22	173	77
5e88989304bf0cde12cf8a2e6c9c5057	<i>Acetobacteraceae</i>	Symbiont	13	60	18	16
615351b38041069cae7587f6f11d7e2f	<i>Lactobacillus helsingborgensis</i>	Symbiont	0	0	13	0
7ccf0063aba554936f596a61eba2509e	<i>Bartonella apis</i>	Symbiont	0	0	0	3
7fc460b386431099c45986947f6d800f	<i>Bifidobacterium asteroides</i>	Symbiont	26	26	24	13
95e79d59da51887288b04f13cd68a88e	<i>Lactobacillus</i> sp.	Symbiont	2	6	7	6
a310dd8d6ac8e59532216a87b485e069	<i>Lactobacillus apis</i>	Symbiont	13	63	200	175
a51341f0383360824e05da5bb63d2f80	<i>Bombella apis</i>	Symbiont	0	216	24	58
add077c21c9d0c2e6d5a9c3073a9f2ff	<i>Bartonella</i> sp.	Symbiont	0	3	7	0
af8df7f3730061d26b9655ec5b4a5c25	<i>Bifidobacterium coryneforme</i>	Symbiont	0	0	6	2
b7420af7805b65881f319c0276233813	<i>Lactobacillus kullabergensis</i>	Symbiont	9	22	0	16
be33fe4f622661dee912ccfbeat7c189	<i>Lactobacillus melliventris</i>	Symbiont	26	7	11	11
c386dac39b3ebd27095ac8c251446b5c	<i>Snodgrassella alvi</i>	Symbiont	11	0	0	0
caa792085f9da84dffef5d8a536329d5	<i>Frischella perrara</i>	Symbiont	23	0	8	4
e0f189a223266cebe35ecce782442771	<i>Lactobacillus mellis</i>	Symbiont	0	13	0	0
e4d48ac9b1335045533830ed6d8617ac	<i>Bombella</i> sp.	Symbiont	0	190	396	497
fda0746dace4494eb6cfd64b8b7d6036	<i>Providencia</i> sp.	Opportunisti c	0	42	0	0

Data S1: Cuticular hydrocarbon data. Amount (ng) of each cuticular hydrocarbon extracted from every sample.

Data S2: 16S rRNA sequencing data. Number of reads sequenced for each ASV in every sample. Taxonomic classifications for abundant ASVs as determined by the Greengenes database (45) (“Greengenes classification”) and by NCBI BLAST (“Likely microbe”).

Data S3: *G. apicola tuf* gene pairwise alignment data. Sheet 1: Proportion of sequence overlap in the *tuf* gene for every *G. apicola* strain sequenced in Fig. 4. Sheet 2: Sample information.

Data S4: Acceptance assay data. Behavioral scoring (accepted/rejected) for all intruder bees used in acceptance assays in Figs. 3 and 4.