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Who is the host of the host-associated microbiome? Colony-level dynamics overshadow individual-level characteristics in the fur microbiome of a social mammal, the Egyptian fruit-bat

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Abstract

In the first longitudinal study of bat microbiomes, we find that unlike the pattern described in humans and other mammals, the prominent dynamics in Egyptian fruit bats' fur microbiomes are those of change over time at the level of the colony as a whole. Thus, on average, a pair of fur microbiome samples from different individuals in the same colony collected on the same date are more similar to one another than a pair of samples from the same individual collected at different time points. This pattern suggests that the whole colony may be the appropriate biological unit for understanding some of the roles of the host microbiome in social bats' ecology and evolution. This pattern of synchronized colony changes over time is also reflected in the profile of volatile compounds in the bats' fur, but differs from the more individualized pattern found in the bats' gut microbiome.

Keywords: fruit bats ; microbiome ; pathogens ; ecology ; behavior

Introduction

The host-associated microbiome may play a role in the host's fate on many timescales, from the short-term health and behavior of the individual ((1–8)), through the life-long ecology and life history of the animal ((9)), to the long-term evolutionary adaptation of a species to its environment ((10–12)). However, data regarding non-human vertebrate microbiomes, particularly those of skin or fur, are just beginning to accumulate, and our understanding of the processes that determine their composition and function is limited.

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41 This is particularly true for non-model organisms: to date, few studies have collected

42 longitudinal samples of non-human microbiomes in ecologically-realistic settings ((13)).

43   In this article we report on the temporal dynamics of the fur and the gut microbiome,

44 assessed using 16S rRNA gene amplification, of ten Egyptian fruit bats (*Rousettus*

45 *aegyptiacus*) in a captive colony (henceforth, the 'experimental colony'). The colony was

46 sampled weekly over a period of 13 weeks, in addition to sparser sampling of all 33

47 individuals in this colony and of a few additional individuals in a non-captive colony over

48 the same time period (henceforth the 'open colony'). We compare these data to the volatile

49 components found in the bats' fur, collected weekly and analyzed using gas chromatography

50 (*GC*).

51   Our findings allow us to address an on-going debate regarding the conceptualization of

52 host-microbiome ecology and evolution: some perspectives emphasize the potential utility

53 of a holobiont theory, which regards the host and its associated microbial species together as

54 a meaningful ecological and evolutionary unit (14, 15). Others focus on a metagenomic

55 function-oriented account of the host and its associated microbiota (16, 17). Yet others

56 suggest that no such novel theory is required, and that the ecology of the host and its

57 associated microbiome can best be understood in terms of existing evolutionary and

58 ecological theory, e.g. microbe-environment interaction or generalized multi-species Lotka-

59 Volterra dynamics (18). This debate also recalls an earlier debate in evolutionary biology

60 about levels of selection and ecological dynamics, with different perspectives suggesting the

61 gene, the individual, the kin group, or the social group as the meaningful biological unit,

62 whose trajectory in time is most useful and meaningful to track (19, 20). The different

63 perspectives are not mutually exclusive, however, and may contribute complementary

64 insights.

65   Our empirical findings in the bats' fur microbiome suggest a perspective that to the best

66 of our knowledge is new, and which informs both the conceptualization of the host-

67 associated microbiome and the question of the level at which ecological dynamics take

68 place. We propose that in some cases the whole colony of host organisms functions as a

69 collective host with which the microbiome is associated.

70   A range of studies in humans and a few in wild animals have suggested that at certain

71 body sites, such as the gut or skin, the primary determinant of the microbiome composition

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72   is individual identity (21–29). That is, on average, two microbiome samples from the same

73   individual, taken at different time points, will be more similar to one another in their

74   composition than two samples from different individuals, even for individuals controlled for

75   sex, age, and other variables. Here we report that this regularity is not seen in the

76   composition and dynamics of the fur microbiome of a highly social mammal that roosts in

77   tight colonies – Egyptian fruit bats. Instead, we find that changes over time in the fur

78   microbiome are best described as occurring at the colony level, with inter-individual

79   variation playing a secondary role. The pattern seen in the gut microbiome, however, is

80   different: some coordinated change in microbiome composition occurs, but this

81   phenomenon is secondary to the role of individual identity and sex in determining

82   individuals' gut microbiomes.

83   Dynamics of change over time occur in the bats' fur chemistry as well: the bats' fur

84   constitutes a habitat whose conditions strongly influence the composition of the

85   microbiome, and are also affected by it. The idea that an animal's microbiome will shape its

86   odor and will thus play a role in its sociality (e.g., via olfactory recognition) has been raised

87   multiple times, but studied mostly with respect to the microbiome in scent glands or

88   specialized organs involved in olfactory communication ((30–36)). As with the composition

89   of the microbiome, our results suggest a colony-level change over time of the bats' fur

90   chemistry. In addition, certain microbial taxa are linked to changes in the furs' profile of

91   volatile compounds.

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93   **Results**
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95   A total of 518 samples of the fur and gut microbiota of bats, together with 36 samples of

96   food and environmental control samples, were analyzed in this study, yielding 7196 non-

97   chimeric Operational Taxonomic Units (OTUs) at 99% identity. These could be assigned

98   (Figure 1) to 581 bacterial species, belonging mainly to the phyla Firmicutes (mean relative

99   abundance in fur: 46%, and in gut: 39%) and Proteobacteria (mean relative abundance in

100   fur: 30%, in gut: 38%). The near absence of Bacteroidetes, usually prevalent in animal guts

101   (37), is noteworthy and likely related to the fruit bats' unique habits and diet. To minimize

102   weight during flight, bats chew the fruit, ingesting the juices and discharging much of the

103   remaining pulp; ingested fruit passes rapidly through the digestive tract, with a short

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104   retention time of ~40 minutes (38, 39). The fruit bats' diet thus relies heavily on

105   consumption of simple sugars; accordingly, the intestine is relatively short, undifferentiated

106   and with no observed cecum. It seems quite possible that this short time, and the non-

107   compartmental structure of the digestive tract, do not allow appropriate conditions for the

108   anaerobic fermentation of complex sugars that the Bacteroidetes typically carry out. Fur

109   samples were far more diverse than gut samples (mean Shannon index 5.15, as opposed to

110   5.84, p<2.2e-16), yet there was also a high degree of overlap between the communities, with

111   *Streptococcus salivarius* (mean abundance in fur: 16%, in gut: 13%) being the most

112   common species in both sites. The microbiome composition in samples from the

113   experimental colony was comparable to that of the open colony, a colony of fruit bats that

114   roost in our cave-like facility, but behave as wild bats, flying nightly out to forage (Figure 1,

115   and see below).

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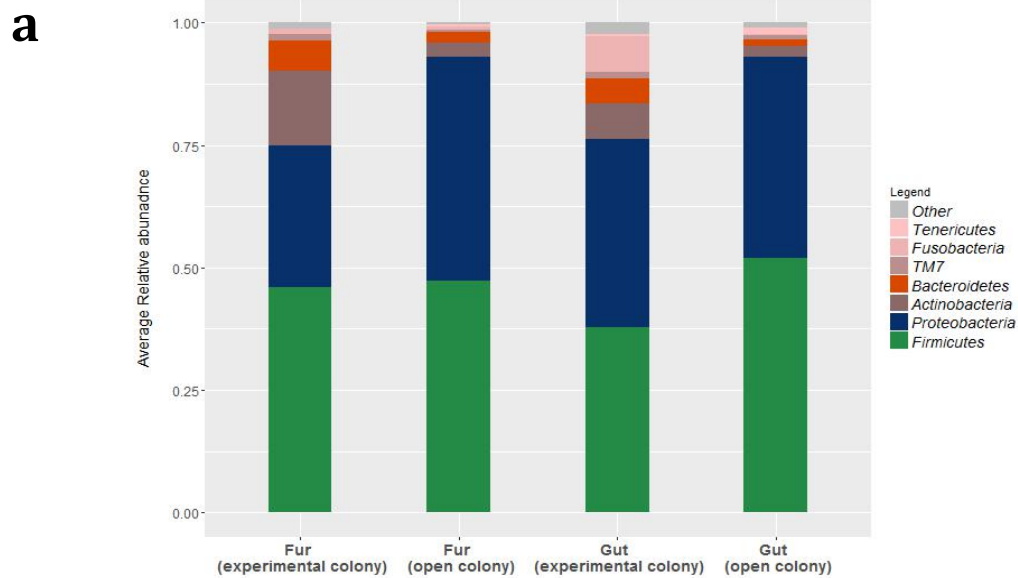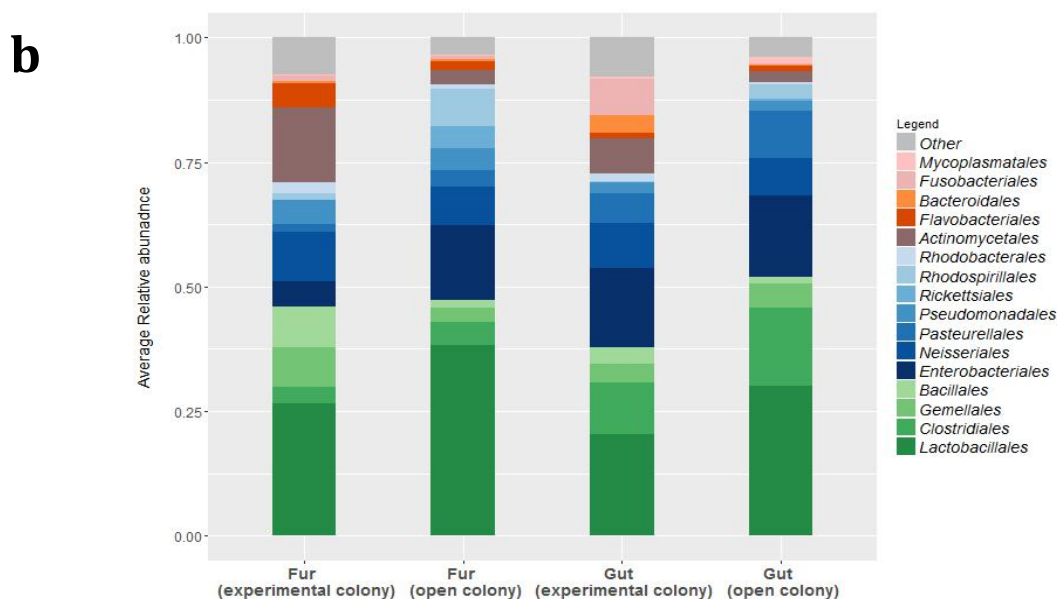
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Figure 1. Microbial composition of fur and gut samples. The average relative abundance of each taxon per site is shown. All taxa with a mean relative abundance < 1% were grouped to a single category named "Other". (a) Phylum level; (b) Order level; orders belonging to the Proteobacteria phylum are in shades of blue; orders belonging to the Firmicutes phylum are shown in shades of green.

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125   The most striking observation, and the focus of this report, was a coordinated change in

126   fur microbiome composition over time across all individuals (**Figure 2a**)[1]. This pattern is

127   strong relative to the lack of visual clustering of the samples according to individual identity

128   (**Figure 2b**). A permutation analysis of variance supports this visual observation: the date on

129   which each sample was taken explains circa 35% of the fur microbiome variance in the

130   experiment colony, while individual identity explains only circa 8% of the variance

131   (PERMANOVA test using the Adonis method in R, $p<0.001$). To validate this finding, we

132   conducted a comparison of the distances between pairs of samples. We found (**Figure 3a**)

133   that samples from different individuals on the same date are, on average, more similar to

134   one another than samples taken on different dates but from the same individual (Kruskal-

135   Wallis test, p-value<0.001; confirmed using a Mantel test to avoid pseudo-replication, p-

136   value<0.001; See analogous analyses with other distance measures in Supplementary

137   Section 3).

138   This finding is supported by a number of additional analyses (see Supplementary

139   Sections 2 and 3): (1) Qualitatively similar results are obtained when using different

140   weighted and unweighted distance measures (Binary, Bray-Curtis, Jaccard, Jensen-

141   Shannon divergence, unweighted Unifrac, and Weighted Unifrac), and considering

142   different bacterial taxonomic levels. (2) The pattern of coordinated change of the fur

143   microbiome across the whole colony was even clearer when we included samples from the

144   entire colony. The colony consisted of 33 individuals, each of which was sampled once at

145   the beginning of the experiment and again at its end. (3) Results were robust to multiple

146   conservative data filtering schemes, which ensured that possible bacterial contamination

147   had been removed from the dataset (see Methods and Supplementary Section 2).

148   These colony-level changes over time are not easily explained by the study of the

149   dynamics of particular microbial taxa (see examples of such dynamics in supplementary

150   Section 4). Instead, the colony-level dynamics seem to be an emergent property of the host-

151   microbiome system as a whole. The dynamics can be observed most clearly when

152   considering the overall composition of the bats' microbiomes. They are most obvious when

153   the microbiome composition is measured in terms of only the presence or absence of each

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[1] The bacterial community in each sample was characterized using multiplexed 16S rRNA gene amplicon sequencing. See Methods.

154   taxon and not their relative abundances, suggesting that a prominent part of the change in

155   time occurs in microbial species that are generally found at low frequencies (see
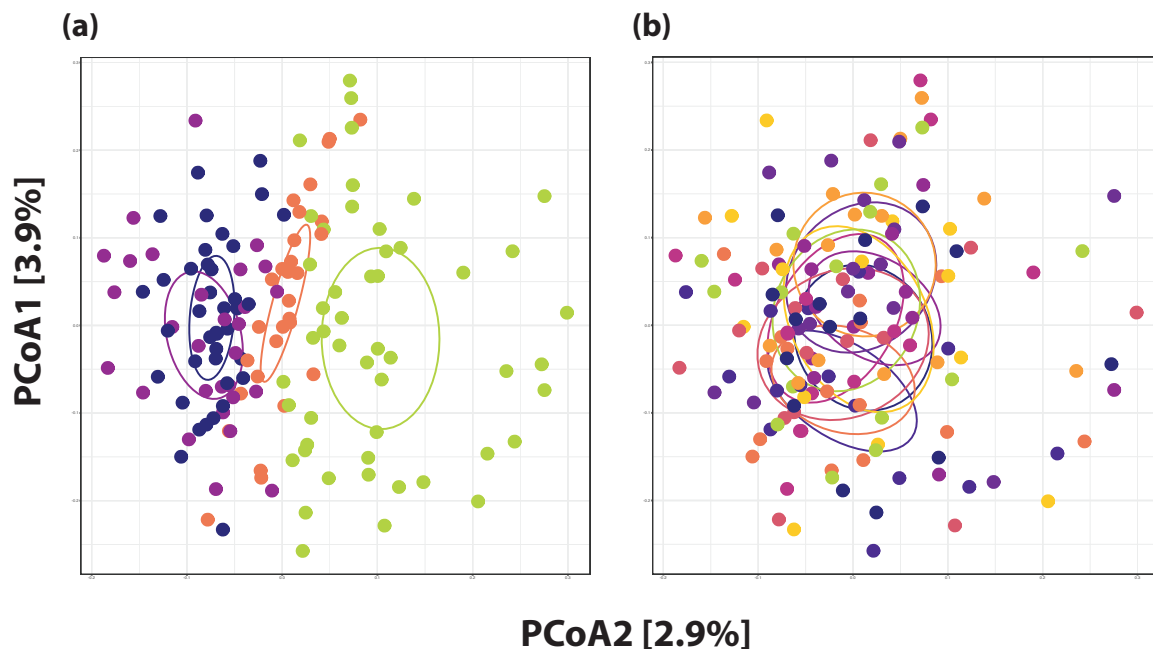
156   Supplementary Section 2).

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160   **Figure 2: The prominent pattern in the fur microbiome is that of colony-level**

161   **change over time.** Samples from the fur of the ten focal bats in the experiment colony

162   (weekly samples, over 13 weeks), plotted using PCoA of the Jaccard distance between

163   samples. Each point represents a sample. In **(a),** each sample is colored according to its

164   date of sampling; dates are divided into the four time quarters of the 13-week period of

165   the experiment (quarters 1-4 are denoted by blue, purple, orange, and green,

166   respectively). The clustering is seen to correspond to the quartile of sampling. In **(b)**,

167   each sample is colored according to the individual bat from which it was taken. No clear

168   clustering according to individual identity is visually apparent. Here and in all other

169   PCoA plots, each ellipse represents the region around the center of mass of the samples
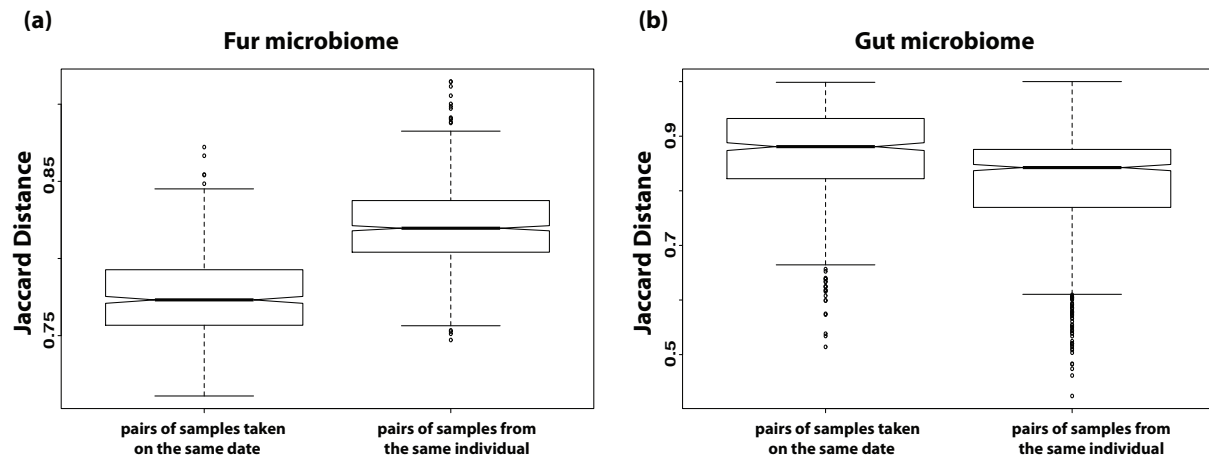
170   in the group (see Methods).

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175 **Figure 3: Similarity of pairs of samples in the experiment colony, from the same**

176 **individual or from the same date.** In the fur microbiome **(a)**, pairs of samples from

177 different individuals, collected on the same date, are more similar than pairs of sample

178 from the same individual from different dates. In the gut microbiome **(b)**, pairs of gut

179 samples from different individuals, collected on the same date, are less similar than pairs

180 of samples from the same individual on different dates. Box plots show the median and

181 distribution of the Jaccard distance between all pairs of samples in each of these

182 categories; hinges represent distribution quartiles, and notches the 95% confidence

183 intervals of the medians. Differences between all distributions are highly significant

184 (Kruskal-Wallis test, p<0.0001).

185

186 Fur microbiome samples from the open colony, collected on the same dates, do not share

187 the temporal trajectory of the experimental colony, ruling out the possibility that the inter-

188 individual similarities result from artifacts in the collection or sequencing processes

189 (Supplementary Section 1). The samples from the open colony do, however, recapitulate the

190 pattern found in the experiment colony: they show a significant colony-level change over

191 time, supporting the generality of the finding and showing that it is not an artifact of

192 captivity (Supplementary Section 1, Figures S1.1-S1.2; sample date explains 60% of the

193 variance and individual identity explains 10% of the variance according to PERMANOVA;

194 p<0.001). The fur microbiomes of the bats in the two colonies were similar: 96% of the

195 species found in the open colony were also found in the experiment colony. However,

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196   microbial alpha diversity was somewhat higher in the experiment colony, with mean

197   Shannon index of 6.39 in the experimental colony, and 5.97 in the open colony (a

198   statistically significant difference: two-tailed t-test, $p < 0.001$; calculated on presence/absence

199   data, at the OTU level, on the dataset cleaned from potential contaminants; see additional

200   measures and further details in supplementary Section 6).

201   A parallel analysis of the gut microbiomes yielded a different pattern: although sampling

202   date was found to be a statistically significant explanatory variable, explaining circa 10% of

203   the variance among samples (PERMANOVA, $p < 0.001$), it was secondary to individual

204   identity, which explained approximately 30% of the variance (PERMANOVA, $p < 0.001$).

205   Accordingly, in agreement with the findings reported for a range of body sites in humans

206   and other vertebrates (24, 25, 29, 40), pairs of gut microbiome samples from the same

207   individual are more similar to one another than pairs of samples from the same day but from

208   different individuals (Figure 2b; Kruskal-Wallis rank sum test, p-value< 0.001; confirmed

209   using a Mantel test to avoid pseudo-replication, p-value< 0.001).

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211   The difference between the main factors driving the dynamics in the two body sites, date

212   in the fur and individual in the gut, highlights the colony level dynamics as a feature not of

213   the bat microbiomes in general, but of the bat fur microbiome specifically. This is true

214   despite the fact that the diet of all individuals in the captive colony was almost identical, a

215   factor that should have increased the similarity of individuals' gut environments and

216   therefore their microbiomes.

217   The fur microbiome is expected to be strongly influenced by the fur chemistry, and also

218   to influence that chemistry. To examine the correlation between fur microbiome and fur

219   volatiles, we collected fur samples from the experimental bats every two weeks and

220   analyzed the composition of their volatile molecules by gas chromatography (GC). We

221   found a pattern analogous to the one seen in the fur microbiome: the prominent factor

222   governing variability is a change in the volatile profile over time, which is common across

223   individuals (**Figure 4a**; Adonis PERMAONVA, variance explained: 27%, $p < 0.001$). As in

224   the case of the microbiome, individual identity is less important in explaining the

225   composition of samples and it does not reach a significance threshold in a PERMANOVA

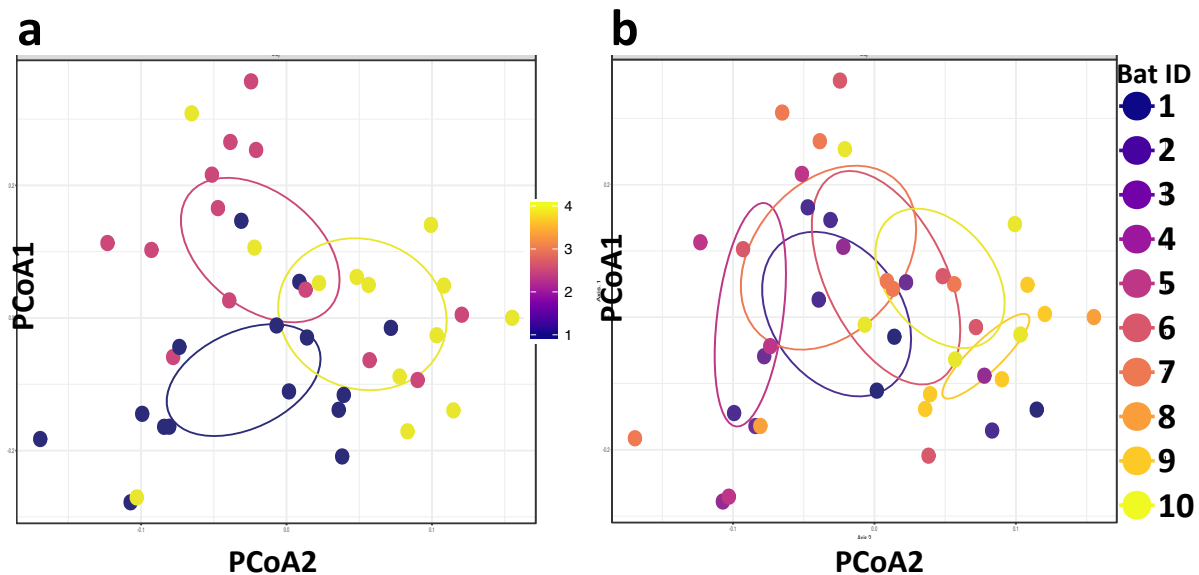226   test ($p = 0.43$; see also **Figure 4b**).

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228 **Figure 4: Colony and individual-level patterns in volatiles' profiles.** Samples of the

229   profile of volatile compounds in the fur of the ten focal bats in the experiment colony,

230   plotted using PCoA of the Bray-Curtis dissimilarity measure between samples. Each

231   point represents a sample. **(a)** Samples are colored according to collection date; dates are

232   divided into the three time trimesters of the 13-week period of the experiment

233   (trimesters 1-3 are colored blue, red, and yellow, respectively). Only 3 periods (vs. 4 for

234   the microbiome) were used for volatile analysis due to the smaller number of samples.

235   **(b)** Samples are colored according to the individual from which they were collected.

236   Ellipses represent the areas around centers of mass (see Methods).

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238   The concentrations of a number of volatile compounds were significantly correlated

239 with the abundance of certain bacterial taxa in the fur, which are known producers of these

240 volatile metabolites: Cholestane diene was significantly positively correlated with three taxa

241 of the order Actinomycetales (genera *Nesterkonika*, *Arthrobacter* and *Brevibacterium*).

242 Palmitic acid was significantly correlated with the genus Neisseria. Oleic acid was

243 significantly correlated with the genera *Alkanindiges* and *Neisseria*. All three compounds

244 are known to play a role in communication among vertebrates (see supplementary section

245 5). This suggests that some of the change in volatiles over time might reflect the respective

246 colony-level changes in the fur microbiome (the volatile dynamics remained significantly

247 dependent on time even when the dataset is reduced to include only these bacteria-related

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248   volatiles: Adonis PERMANOVA, variance explained: 20%, p<0.005,). It is likely that the

249   two modalities – fur chemistry and microbiome composition – interact with one another.

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253 **Discussion**

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255   Unlike findings in other vertebrates, the microbiome of the fur of the Egyptian fruit-bat

256 changes over time in a manner that is coordinated across the whole colony; this coordination

257 is the prominent driver of variation in our data. Why are the temporal dynamics of the

258 Egyptian fruit bat fur microbiome different from those of microbiomes in other studied

259 mammals (e.g. (26, 29))? We propose that the frequent physical interactions between the

260 bats in a colony (the bats perch in very tight clusters) have a homogenizing effect on their

261 fur microbiomes, leading to dynamics in which the fur microbiomes of all individuals in the

262 colony function together as a single ecosystem or super-organism. The changes over time

263 may be driven by external factors, such as changes in the physiological state, in diet, or

264 seasonal changes (although such changes were largely controlled in our experiment; see

265 supplementary Section 8), but also by processes that are "internal" to the bacterial

266 community such as neutral drift, local adaptation, and ecological succession.

267   Similar dynamics to those we find have been described in datasets from individuals

268 along a developmental trajectory such as studies of human infant microbiomes (41–43),

269 suggesting an ecological succession process, driven by physiological maturation of the host.

270 The vast majority of individuals in our study were fully mature, so this cannot be the

271 underlying driver of the pattern we see. However, a physiological change of that nature may

272 account for some of the microbiome change over time in our experiment colony: for

273 example, changes in the females' reproductive state, which were correlated across most

274 females and that became pregnant at about the same time, accounted for 4% of the

275 microbiome variation (PERMANOVA, Adonis method in R, $p<0.0001$).

276   Our second main finding is that the gut microbiome is not characterized by such a

277 prominent change in time as seen in the fur. Why are the dynamics of the fur and the gut

278 microbiomes so qualitatively different? One possibility is that the difference is due to the

279 relative role that common environmental factors play in each of these two modalities: the fur

280 environment is strongly influenced by external factors, while the gut environment is

281 strongly affected by the individual's physiology and immune system, which buffer it from

282 such environmental influences as diet, which is largely common to all individuals in our

283 colony. This buffering can be seen as adding a "personalizing" effect, increasing the role of

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284   individual identity determining gut microbiome composition. Another possibility is that the

285   different dynamics stem from differences in dynamics of bacterial transmission: the bats'

286   behavior, which includes frequent and extensive physical contact, has a homogenizing effect

287   on the fur microbiomes, a process from which the gut microbiome is relatively shielded.

288   From the bacterial perspective, one can think of gut bacteria as facing a greater transmission

289   limitation than fur bacteria, creating a structured meta-population in which each individual's

290   gut constitutes an "island", allowing both neutral and selectively-driven divergence between

291   the microbiomes in different guts. These possibilities are not mutually exclusive.

292   The functioning of the colony's fur microbiome as a single, highly connected, ecosystem

293   might have important implications on the behavior and ecology of bats and other social

294   species that roost in close proximity. Analysis of the volatiles found on the bats' fur suggests

295   that the fur microbiome may play a role in maintaining the social structure of the colony by

296   facilitating olfactory-based recognition of colony members. Analysis of the volatiles in the

297   two colonies (experimental and wild) at a single time point revealed that the two differ (see

298   supplementary Section 5).

299   Bats' associated microbes have recently received much attention from two specific

300   perspectives: the first views bats as potential reservoirs of zoonotic pathogens that may

301   infect humans (44–47). The second focuses on the pathogens of the bats themselves,

302   particularly on dynamics of the white nose syndrome (48, 49) a serious emerging bat

303   epidemic in bats (50, 51). The highly correlated dynamics of the colony members' fur

304   microbiomes suggests that in bats, and perhaps more broadly – in social species that roost in

305   great proximity – the resilience to some types of disease may be largely a colony-level trait,

306   and less a feature of individuals. This has obvious implications, potentially influencing plans

307   for intervention that would mitigate the effects of the white nose syndrome or minimize the

308   prevalence of specific zoonotic pathogens.

309   From a more theoretical evolutionary standpoint, our findings suggest that selective

310   pressures on and through the fur microbiome, in species that are characterized by frequent

311   physical contact between individuals, may act most prominently at the colony level, and not

312   at the level of the individual, as is commonly assumed. This implies that it may be highly

313   informative to supplement the study of host-microbiome dynamics with a meta-community

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314 framework that incorporates hierarchically structured transmission dynamics and in which

315 colonies are the entities whose fate is studied.

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318 **Methods**

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320 **Data collection**

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322 Two major colonies of bats are held in the Tel Aviv University zoological garden facility.

323 The first, denoted *the experimental colony*, consisted of 33 bats at the time of this study. The

324 second, denoted the *open colony*, consisted of 35 free ranging bats that can fly out and come

325 back as they wish. From the experimental colony, the same 10 focal bats, 5 males and 5

326 females, were sampled once a week for their gut and fur microbiome during March to June

327 2016. Additionally, 4 focal bats from the open colony were sampled at 10 time points for

328 comparison (not all were present in all 10 time points, as happens in a free ranging bat

329 colony); mean number of samples from each bat is 7).

330 All bats were handled with single use clean gloves and swabbed for DNA before other

331 measurements were taken, in order to limit contamination. The samples were taken by

332 sterile culture swab applicators (BD CultureSwab™) moistened with Ringer's Solution. Fur

333 sampling was done by sweeping the swab, back and forth, 10 times over each of four

334 different sites: shoulders, arm pits, stomach and muzzle. Sampling the gut microbiome was

335 done by holding the bat and squeezing the anus to extract transparent discharge. This

336 discharge was collected by sterile culture swab applicators moistened with Ringer's

337 Solution. *Rousettus aegyptiacus* has a relatively short intestine, not differentiated into small

338 and large parts and with no observed cecum or appendix (38); the duration of the intestinal

339 pass is approximately 40 minutes (38, 39). As the bats were after their day-fast and the

340 intestine was free of content, we suggest that this discharge well represents the core gut

341 microbiome without using invasive or lethal techniques (see supplementary Section 6 for a

342 comparison of the microbiome in these samples and in those found in the bats' feces). All

343 bats were sampled in the same way and in the same order. Additional environmental

344 samples were collected from the fresh food plates, capture nets, and air. After sampling, the

345 swabs were sealed in a sterile plastic container provided, and immediately taken for DNA

346 extraction.

347 **DNA extraction and pyrosequencing**
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349 Genomic DNA was extracted from swabs using the PowerSoil© DNA isolation Kit
350 (MoBio Laboratories), as recommended by the manufacturer. Extracted DNA samples were
351 stored at −20°C. PCR amplification of the 16S rRNA gene was carried out with universal
352 prokaryotic primers containing 5-end common sequences
353 (CS1-341F   5'-ACACTGACGACATGGTTCTACANNNNCCTACGGGAGGCAGCAG
354 and CS2-806R 5'-TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT).
355 Twenty eight PCR cycles (95°C 15 sec., 53°C sec. 15, 72°C 15 sec.) were conducted using the
356 PCR mastermix KAPA2G Fast™ (KAPABiosystems); successful amplification was verified
357 by agarose gel electrophoresis. Sample-specific barcodes and Illumina adaptors were added
358 in 8 additional PCR cycles, and paired-end deep sequencing of the PCR products was
359 performed on an Illumina MiSeq platform at the Chicago Sequencing Center of the
360 University of Illinois. Sequencing depth ranged from 1589 to 30000 sequences per sample;
361 to ensure data evenness, data were rarefied to an equal depth of 1500 sequences per sample.
362
363 **Data analysis**
364 Demultiplexed raw sequences were quality filtered (PHRED quality threshold <20) and
365 merged using PEAR (52). Sequences shorter than 380bp (after merging and trimming) were
366 discarded. Data were then analyzed using the Quantitative Insights Into Microbial Ecology
367 (QIIME, version 1.9) package (53) in combination with VSEARCH (54). Sequences were
368 de-replicated and ordered by size before OTU clustering at 99% threshold; to reduce
369 spurious formation of OTUs, singleton sequences were not allowed to form new OTUs.
370 Chimeric OTUs were detected and discarded using UCHIME (55) algorithm against the
371 gold.fa database. Taxonomy was assigned using UCLUST (56) against the QIIME default
372 database (greengenes 13.8) .
373
374 Analysis downstream from QIIME was done in R and in Matlab. Primary R packages
375 used were Phyloseq, Vegan and Caret. Statistical tests were conducted using their
376 implementation in these packages, with the following settings: **PERMANOVA**:
377 Adonis{vegan}, permutations = 10,000. **Mantel Test**: mantel{vegan}, method = Pearson,
378 permutations =   10,000. **Linear Discriminant Analysis (LDA)**: lda{caret}. In the

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379   PERMANOVA tests no strata were used, and the effect of each variable, e.g. date of

380   sampling, individual identity, sex, and age, was assessed separately. Note that some of these

381   variables are co-linear. This procedure does not control for pseudo-replication, and thus a

382   Mantel test was conducted to support assertions regarding significance of variables

383   wherever possible, and PCoA clustering was used for visual demonstrations. PCoA plots

384   were made using ordinate{phyloseq}, with the default settings. The ellipses which describe

385   each group's center of mass are used for ease of visualization of the center of the

386   distribution of the points in that group, and reflect the 25% confidence level around the

387   center of a fit of these points to a multivariate normal distribution. Mantel tests were used to

388   assess whether pairs of samples from the same individual and those from the same date are

389   more or less similar to one another; this was done by performing a Mantel test on the matrix

390   of Jaccard distances and the matrix obtained by assigning 1 to pairs of samples from the

391   same date and 2 to pairs of samples from the same individual (see also (29)).

392   All results in the main text from Figure 2 onwards are for the dataset composed of the

393   focal individuals only, following the most conservative procedure of omitting potential

394   contaminant taxa. This included the removal of all microbial taxa that occurred in the

395   negative controls or in more than one of the samples of the bats' food at a frequency above

396   0.2%. The samples from the food were collected before it was introduced into the colony,

397   and thus any microbial taxa in them were viewed as potential contaminants. This procedure

398   may have omitted taxa that were not contaminants, and so the analyses were repeated with

399   the full dataset as well, to confirm that they yield the same qualitative results. Wherever

400   meaningful, analysis with the full range of samples is included in the supplementary

401   material. PERMANOVA tests and LDA analysis were done using the matrix of relative

402   abundances of microbial taxa, and PCoA plots in the main text present Jaccard distances

403   based on presence/absence of microbial taxa. Analogous analyses with additional distance

404   measures are presented in the supplementary material.

405

406   **Analysis of volatile compounds in fur using gas chromatography**

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408   Fur samples were placed in 3ml vials containing dichloromethane, for a minimum of 7

409   days. The samples were sieved, extracts were transferred to new insert vials while the fur

410   was removed, dried and weighed for each sample. Two internal standards (udecanal and

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411 ergosterol) of known concentration (0.01 ng/μL) were added to each extract. Samples were

412 first analyzed by combined gas chromatography/mass spectrometry (GC-MS;GC 7890A,

413 MS 5975C; Agilent) using an HP-5MS capillary column, that was temperature programmed

414 from 60°C to 300°C at 10°C/min. Compounds were identified by their mass fragmentation

415 and retention times compared with synthetic standards when available. Compound

416 quantification across samples was thereafter performed by gas chromatography with flame

417 ionization detection (GC-FID) (CP 3800; Varian) using a DB-1 fused silica capillary

418 column (30 m × 0.25 mm i.d.), temperature programmed as above, using peak integration.

419 22 peaks in the normalized chromatograms (Supplementary Section 5) were identified

420 using GC-MS as biological compounds (rather than artificial contaminations). After

421 removal of samples that failed to produce data, this process resulted in a matrix of 22 by

422 41 representing 22 volatiles sampled from 10 individuals over 6 time points (19 samples

423 yielded no peaks probably because too little fur was collected and thus we had 41 and not

424 60 samples). Analysis of the resulting dataset was executed, for consistency, using the same

425 methods and scripts as used for the PCoA and PERMANOVA analyses of the microbiome

426 data. Correlations between abundance of microbial taxa and volatile compounds were

427 carried out at the OTU level. For each of the 22 volatiles, a 41-dimentional vector

428 representing the levels of this volatile across individuals and times was created. This vector

429 was then (Pearson) correlated with a 41-dimentional vector representing the levels of an

430 OTU (sampled over the same individuals and dates). Only the 30 OTUs that appeared in at

431 least 50% of the samples of all individuals were used. This procedure was repeated over all

432 22 volatiles and 30 OTUs resulting in a (30x22) correlation matrix. Significant correlations

433 were chosen following an FDR correction for multiple comparisons.

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435 **Data Accessibility**
436 All data supporting the results reported in this study will be made available upon
437 publication.
438
439 **Acknowledgements**
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448   **References**

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