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Coordinated change at the colony level in fruit bat fur microbiomes through time

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The host-associated microbiome affects individual health and behaviour, and may be influenced by local environmental conditions. However, little is known about microbiomes' temporal dynamics in free-living species compared with their dynamics in humans and model organisms, especially in body sites other than the gut. Here, we investigate longitudinal changes in the fur microbiome of captive and free-living Egyptian fruit bats. We find that, in contrast to patterns described in humans and other mammals, the prominent dynamics is of change over time at the level of the colony as a whole. 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.

The host-associated microbiome may play a role in the host's fate on many timescales, from the short-term health and behaviour 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. This is particularly true for non-model organisms: to date, few studies have collected longitudinal samples of non-human microbiomes in ecologically realistic settings[13].

In this paper, we report on the temporal dynamics of the fur and gut microbiome, assessed using 16S ribosomal RNA (rRNA) gene amplification, of 10 Egyptian fruit bats (*Rousettus aegyptiacus*) in a captive colony (constituting 33 individuals; henceforth, the 'captive colony') and 4 individuals from a wild colony (constituting 20–50 individuals; henceforth, the 'open colony'). The bats in both colonies were habituated to occasional handling, allowing repeated sampling of the same individuals. Each of the 14 focal bats was sampled weekly over a period of 13 weeks, in addition to sparser sampling of the other 23 individuals in the captive colony. We compared these data with the volatile components found in the bats' fur, collected weekly in the captive colony and analysed using gas chromatography.

Our empirical findings on the bats' fur microbiome inform both the conceptualization of the host-associated microbiome and the level at which ecological dynamics takes place. We propose that, in some cases, the whole colony of host organisms functions as a collective host with which the microbiome is associated. Colony-level similarities in the composition of the microbiome have previously been demonstrated in a number of vertebrates (for example, refs [14–20]). Our study provides a longitudinal trajectory of colony-level changes of the microbiome over time in a wild animal.

A range of studies in humans and a few in wild animals have suggested that at certain body sites, such as the gut or skin, the primary determinant of the microbiome composition is individual identity[21–29]. That is, on average, two microbiome samples from the same individual, taken at different time points, will be more similar to one another in their composition than two samples from different individuals, even for individuals controlled for sex, age and other variables. Here, we report that this regularity is not seen in the composition and dynamics of the fur microbiome of a highly social mammal that roosts in tight colonies—the Egyptian fruit bat. Instead, we find that changes over time in the fur microbiome are best described as occurring at the colony level, with inter-individual variation playing a secondary role. However, the pattern seen in the bats' gut microbiomes is different: some coordinated change in microbiome composition occurs, but this phenomenon is secondary to the role of individual identity and sex in determining individuals' gut microbiomes.

Change over time occurs in the bats' fur chemistry as well: the bats' fur constitutes a habitat whose conditions strongly influence the composition of the microbiome, and are also affected by it. As with the composition of the microbiome, our results suggest a colony-level change over time of the bats' fur chemistry. We also find that certain microbial taxa are linked to changes in the furs' profile of volatile compounds. The idea that an animal's microbiome will shape its odour and thus play a role in its sociality (for example, via olfactory recognition) has been raised multiple times, but studied mostly with respect to the microbiome in scent glands or specialized organs involved in olfactory communication[15,18,30–34]. Our results suggest that temporal microbiome dynamics may change individual bats' fur and the colonies' odours over time, potentially influencing their behaviour.

Results

A total of 518 samples of the fur and gut microbiota of bats, together with 36 samples of food and environmental control samples, were

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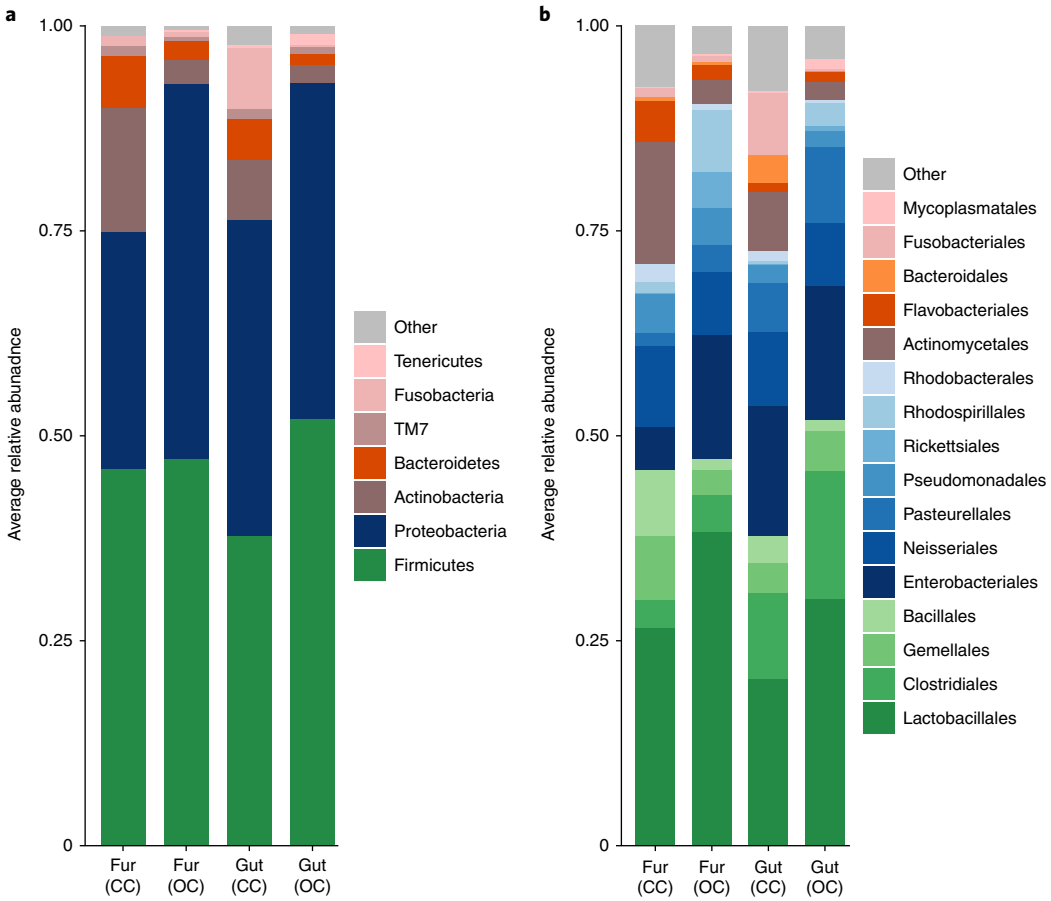

Fig. 1 | Microbial composition of fur and gut samples. Average relative abundance of each taxon per site (fur or gut in the captive colony (CC) or open colony (OC)) at the phylum level (**a**) and order level (**b**). All taxa with a mean relative abundance of <1% were grouped to a single category, 'other'. Orders belonging to the Proteobacteria phylum are shown in shades of blue, whereas those belonging to the Firmicutes phylum are shown in shades of green.

analysed in this study, yielding 7,196 non-chimeric operational taxonomic units (OTUs) at 99% identity. These could be assigned (Fig. 1) to 581 bacterial species, belonging mainly to the phyla Firmicutes (mean relative abundance: 46% in fur and 39% in gut) and Proteobacteria (mean relative abundance: 30% in fur and 38% in gut). Fur samples had higher alpha diversity than gut samples (mean Shannon indices: 5.84 and 5.15, respectively; $P < 2.2 \times 10^{16}$), yet there was also a high degree of overlap between the communities (86% of species were found in both), with *Streptococcus salivarius* (mean abundance: 16% in fur and 13% in gut) being the most common species in both sites (see Supplementary Section 6). The significant overlap between the gut and fur microbiome in both experimental and open colonies may be explained in part by (1) the bats' defecation habits (they often defecate during flight, thus spraying their faeces on the walls of the cave and on other bats) and (2) the bats frequently licking themselves and one another, probably introducing bacteria from the fur into the gut.

The captive colony was kept in a cave-like enclosure ($4.0 \times 2.5 \times 2.5\,m^3$), which, during the sampling period, housed 33 individuals. This was located in the zoological gardens of Tel Aviv University. The open colony was located in a nearby cave-like facility. Its 20–50 inhabitants were free to leave and fly out to forage on a nightly basis (the exact number of individuals changed because the bats came and went). The bats in the captive colony were fed daily ad libitum, while the bats in the open colony were only provided with a very small amount of food to encourage their return to the colony. The bats in the open colony behaved as wild bats, flying out nightly to forage. Global Positioning System tracking of these bats showed that they foraged widely, moving in patterns that strongly resembled bats in nearby wild colonies (Supplementary Fig. 8.1). They encountered bats from other colonies regularly on trees at the foraging sites[35] and often visited nearby wild colonies, typically spending a day or more there before returning to the open colony. In addition, bats from wild colonies visited the open colony on a nightly basis.

Although previous studies have shown that microbiomes of animals in captivity often differ significantly from those in the wild[36–40], we found that the microbiome composition of the captive colony was comparable to that of the open colony, with 96% of the species found in the open colony also found in the captive colony, albeit at different abundances. Microbial alpha diversity was somewhat higher in the captive colony, with mean Shannon indices of 6.39 in the captive colony and 5.97 in the open colony (statistically significant difference by two-tailed *t*-test, $P < 0.001$; see additional details in Fig. 1 and Supplementary Section 6). The high similarity between the two colonies was far greater than has previously been found in other studies of the similarity between captive and wild animals. For example, ref. [37] reports barely any overlap between OTUs found among captive leopard seals and those in the wild, and in ref. [39], approximately 70–75% of OTUs were shared between captive and wild woodrats. The high similarity reported here may be attributable to the frequent addition before the experiment of individuals from the wild to the captive colony (importantly, there was no addition of individuals during the sampling period). A second explanation may be the similarity in diet: the captive colony was fed ripe fruit of domesticated plant species, similar to the fruit for which

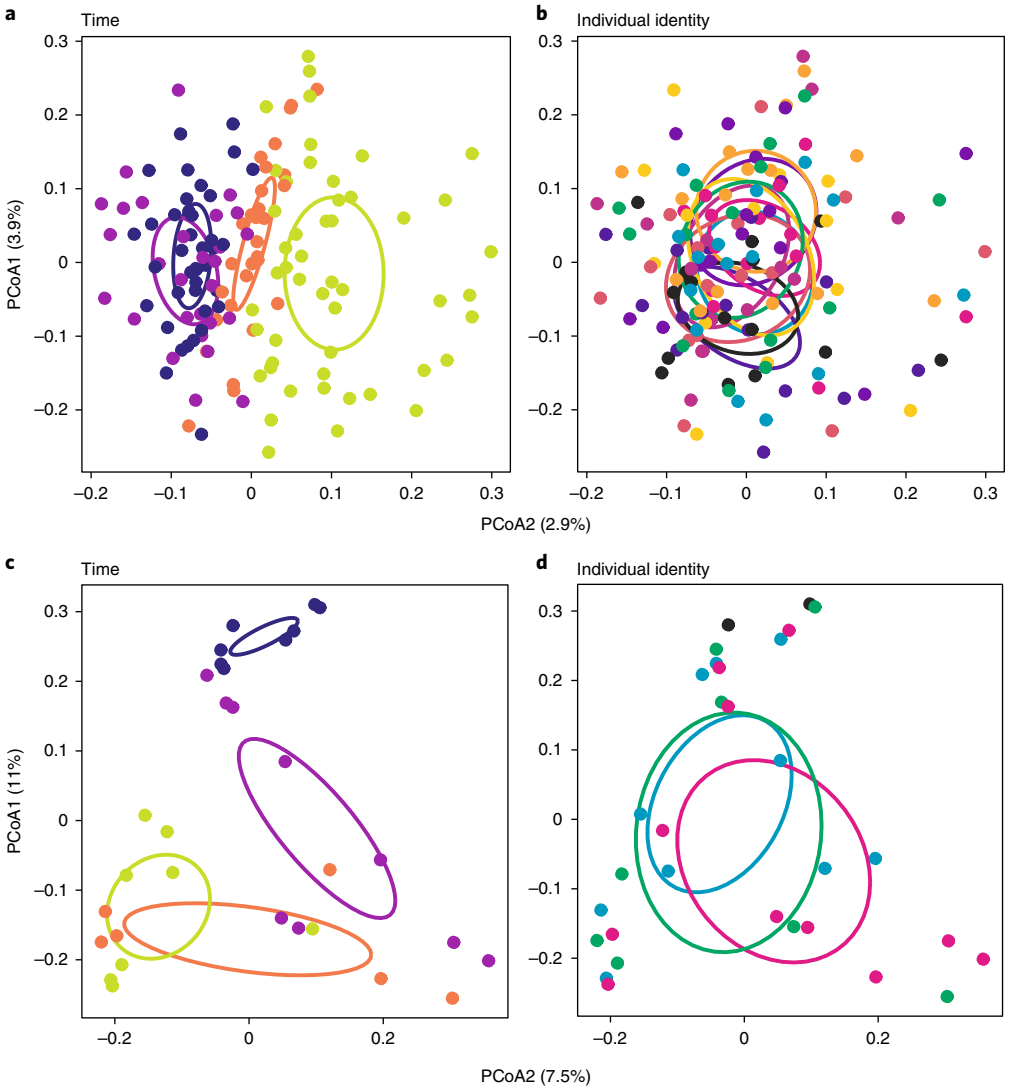

Fig. 2 | The prominent pattern in the fur microbiome is that of colony-level change over time. a–d, Weekly samples (over 13 weeks) were taken from the fur of the ten focal bats in the captive colony (**a** and **b**) and four focal bats in the open colony (**c** and **d**), and plotted using PCoA of the Jaccard distance between the samples. Each point represents a sample. In **a** and **c**, each sample is coloured according to its date of sampling, with dates divided into the four time quarters of the 13-week period of the experiment (quarters 1–4 are denoted by blue, purple, orange and green, respectively). The clustering corresponds to the quartile of sampling. In **b** and **d**, each sample is coloured according to the individual bat from which it was taken. No clear clustering according to individual identity is visually apparent. Here, and in all other PCoA plots, each ellipse represents the region around the centre of mass of the samples in the group (see Methods). Panel **d** lacks a black ellipse due to the small number of samples from the individual represented by this colour.

the wild bats forage in irrigated yards and parks in Tel Aviv and its surroundings.

The most striking observation, and the focus of this report, was a coordinated change in fur microbiome composition over time across all individuals (Fig. 2a,c; the bacterial community in each sample was characterized using multiplexed 16S rRNA gene amplicon sequencing; see Methods). Such a pattern was seen in both the experimental and open colonies. In both, the pattern was strong relative to the lack of visual clustering of the samples according to individual identity (Fig. 2b,d). In addition, we saw the same pattern of coordinated change over time in a longitudinal dataset of fur microbiome samples collected over a similar timescale from nine individuals from the captive colony in the preceding year, during which about one-third of the individuals were different (Supplementary Fig. 1.1).

A permutation analysis of variance supported this visual observation: the date on which each sample was taken explained about 35% of the fur microbiome variance in the captive colony, while individual identity explained only about 8% of the variance (permutational multivariate analysis of variance (PERMANOVA) test using the Adonis method in R, $P<0.001$). The change in time accounted for even more variation among the fur microbiome samples from the open colony: 60% of the variance was explained by the date of sampling, and only 10% was explained by individual identity (PERMANOVA, $P<0.001$). To validate this finding, we conducted a comparison of the distances between pairs of samples from the captive colony. We found (Fig. 3a) that fur microbiome samples from different individuals on the same date were, on average, more similar to one another than samples taken on different dates but from the same individual (Kruskal–Wallis test, $P<0.001$; confirmed using a Mantel test to avoid pseudoreplication, $P<0.001$; analogous analyses with other distance measures are reported in Supplementary Section 3).

Our main finding was supported by a number of additional analyses (see Supplementary Sections 2 and 3): (1) qualitatively similar

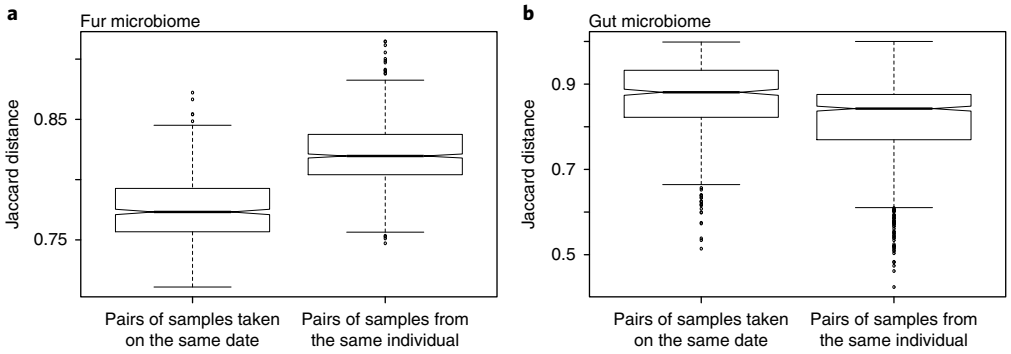

Fig. 3 | Similarity of pairs of samples in the captive colony, from the same individual or from the same date. a, In the fur microbiome, pairs of samples from different individuals, collected on the same date, were more similar than pairs of samples from the same individual on different dates. **b**, In the gut microbiome, pairs of gut samples from different individuals, collected on the same date, were less similar than pairs of samples from the same individual on different dates. Box plots show the median and distribution of the Jaccard distance between all pairs of samples in each of these categories; hinges represent distribution quartiles, and notches the 95% confidence intervals of the medians. Differences between all distributions were highly significant (Kruskal–Wallis test, $P < 0.0001$).

results were obtained using different weighted and unweighted distance measures (binary, Bray–Curtis, Jaccard, Jensen–Shannon divergence, unweighted UniFrac and weighted UniFrac), and considering different bacterial taxonomic levels (Supplementary Figs. 2.1–2.8); (2) the pattern of coordinated change in the fur microbiome across the captive colony was even clearer when we included samples from the entire colony (the colony consisted of 33 individuals, each of which was sampled at the beginning of the experiment and again at its end (Supplementary Figs. 2.1 and 2.2)); and (3) the results were robust to multiple conservative data-filtering schemes, which ensured that possible bacterial contamination had been removed from the dataset (see Methods and Supplementary Section 2; the data presented in Fig. 2 onwards follow the most conservative scheme, in which we removed all OTUs found in negative controls or in more than one sample of the bats' food at a frequency above 0.2%).

These colony-level changes over time are not easily explained by the dynamics of particular microbial taxa (see examples of such dynamics in Supplementary Section 4). Instead, the colony-level dynamics seems to be an emergent property of the host-microbiome system as a whole, and can be observed most clearly in the overall composition of the bats' microbiomes. It is most obvious when the microbiome composition is measured only in terms of the presence or absence of each taxon, and not their relative abundances, suggesting that a prominent part of the change over time occurs in microbial species that are generally found at low frequencies (see Supplementary Section 2).

The finding of a colony-level change in the fur microbiome over time in the captive and open colonies, as well as a separate dataset collected one year earlier in the captive colony (Supplementary Section 1 and Supplementary Fig. 1.1), supports the generality of the finding and suggests that it is not an artefact of captivity, the specific dataset or the particular period during which the samples were collected (Fig. 2, Supplementary Section 1 and Supplementary Figs. 1.1–1.3). Moreover, fur microbiome samples from the open colony, collected on the same dates, did not share the temporal trajectory of the captive colony, ruling out the possibility that the inter-individual similarities resulted from artefacts in the collection or sequencing processes (see Supplementary Section 1; Supplementary Fig. 1.1 shows the separate clustering and independent trajectories over time of the two colonies on a single principal coordinates analysis (PCoA) plot). A linear discriminant analysis (LDA) was used to identify the species that drove the colony-level change over time in each colony; among the ten species that were most important for discrimination between time quarters, only three were common to both colonies (Supplementary Section 4). This further demonstrates that the pattern was not an artefact.

A parallel analysis of the gut microbiomes yielded a very different pattern: although the sampling date was found to be a statistically significant explanatory variable, explaining about 10% of the variance among samples (PERMANOVA, $P < 0.001$), it was secondary to individual identity, which explained approximately 30% of the variance (PERMANOVA, $P < 0.001$; Figs. 3b, 4 and Supplementary Fig. 2.10). Accordingly, in agreement with the findings reported for a range of body sites in humans and other vertebrates[24,25,29,41], pairs of gut microbiome samples from the same individual were more similar to one another than pairs of samples from the same day but from different individuals (Fig. 2b; Kruskal–Wallis rank sum test, $P < 0.001$; confirmed using a Mantel test to avoid pseudoreplication, $P < 0.001$). Notably, individual identity encompassed multiple factors, some of which were common to many individuals, such as sex or age. Much of the variation in the gut microbiomes could be explained by sex.

The difference between the main factors driving the dynamics in the two body sites (that is, date in the fur and individual in the gut) highlights the colony-level dynamics as a feature not of the bat microbiomes in general, but of the bat fur microbiome specifically. This is true despite the fact that the diets of all individuals in the captive colony were almost identical—a factor that should have increased the similarity of individuals' gut environments and therefore their microbiomes.

The fur microbiome was expected to be strongly influenced by the fur chemistry, and also to influence that chemistry. To examine the correlation between fur microbiome and fur volatiles, we collected fur samples from the experimental bats every two weeks and analysed the composition of their volatile molecules by gas chromatography. We found a pattern analogous to that seen in the fur microbiome: the prominent factor governing variability was a change in the volatile profile over time, which was common across individuals (Fig. 5a; Adonis PERMAONVA, variance explained: 27%, $P < 0.001$). As in the case of the microbiome, individual identity was less important in explaining the composition of samples and it did not reach significance in a PERMANOVA test ($P = 0.43$; see also Fig. 5b).

We also explored how the fur chemistry related to the microbiome composition. The concentrations of a number of volatile compounds were significantly correlated with the abundance of certain bacterial taxa in the fur, which are known producers of these

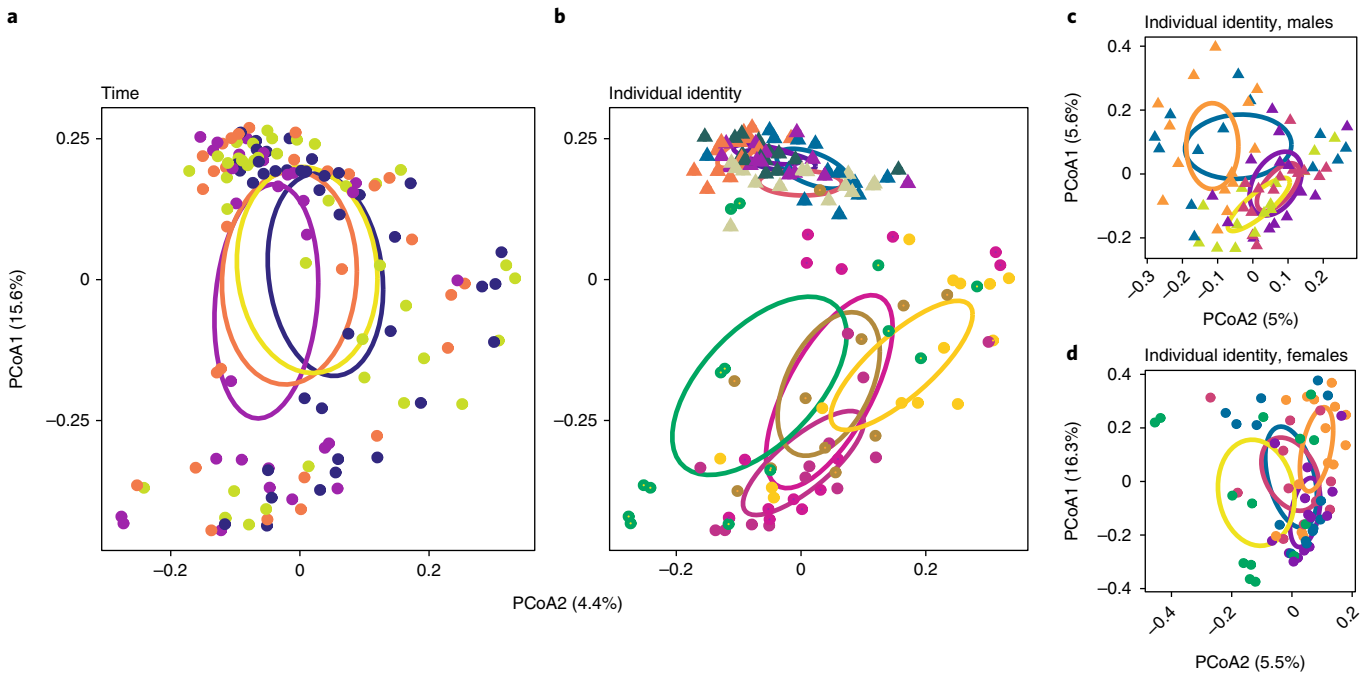

Fig. 4 | In the gut, sex and individual identity are the primary factors that determine microbiome composition. a,**b**, Samples from the guts of the ten focal bats in the captive colony, plotted using PCoA of the Jaccard distance between samples. In **a**, each sample is coloured according to its date of sampling, where dates are divided into the four time quarters of the 13-week period of the experiment (quarters 1–4 are denoted by blue, purple, orange and green, respectively). Some clustering according to the sampling quartile can be seen, but it is secondary to individual identity and sex in its explanation of variation in the data, as shown in **b**, where triangles denote males, dots denote females and each sample is coloured according to the individual from which it was taken. **c**,**d**, PCoAs of the samples collected from males (**c**) and females (**d**) separately. Some clustering according to individual identity can be seen.

volatile metabolites: cholestane diene was significantly positively correlated with three taxa of the order Actinomycetales (genera *Nesterkonika*, *Arthrobacter* and *Brevibacterium*). Palmitic acid was significantly correlated with the genus *Neisseria*. Oleic acid was significantly correlated with the genera *Alkanindiges* and *Neisseria*. All three compounds are known to play a role in olfactory communication among vertebrates (see Supplementary Section 5 and, for example, refs [18,42–48]). This suggests that some of the change in volatiles over time might reflect the respective colony-level changes in the fur microbiome (the volatile dynamics remained significantly dependent on time even when the dataset was reduced to include only these three bacteria-related volatiles (Adonis PERMANOVA, variance explained: 20%, $P<0.005$). It is likely that the two modalities—fur chemistry and microbiome composition—interact with one another. Both modalities are also likely to be strongly influenced by changes in the microbiome of the external environment and changes in the abiotic environment.

Discussion

Unlike findings in other vertebrates, the microbiome of the fur of the Egyptian fruit bat changes over time in a manner that is coordinated across the whole colony; this coordination was the prominent driver of variation in our data. Why are the temporal dynamics of the Egyptian fruit bat fur microbiome different from those of microbiomes in other mammals that have been studied[26,29]? We propose that the frequent physical interactions between the bats in a colony (the bats perch in very tight clusters) have a homogenizing effect on their fur microbiomes, producing dynamics in which the fur microbiomes of all individuals in the colony function somewhat like a single microbiome, with the whole bat colony acting as its host. The changes over time may be driven by external factors, such as changes in the physiological state or diet, or seasonal changes (although such changes were largely controlled for in our experiment; see Supplementary Section 8), but also by processes that are 'internal' to the bacterial community, such as neutral drift, local adaptation and ecological succession.

Similar dynamics to that found here has been described in datasets from individuals along a developmental trajectory such as in human infant microbiomes[49–51], suggesting an ecological succession process, driven by physiological maturation of the host. The vast majority of individuals in our study were fully mature, so this cannot be the underlying driver of the pattern we see. However, a change in mature individuals' physiology of this nature may account for some of the microbiome change over time in our captive colony: for example, changes in the females' reproductive state, which were correlated across most females that became pregnant at about the same time, accounted for 4% of the microbiome variation (PERMANOVA, Adonis method in R, $P<0.0001$). Another process that could have given rise to a succession-like pattern is stress-related changes in individuals' microbiomes as a result of being handled in the experiment itself. Although this is a valid concern, we suggest that it can confidently be rejected as the underlying driver of the observed patterns. If patterns were stress related, we might expect that they would be largely idiosyncratic among bats, and not coordinated among them over time. A coordinated pattern with respect to stress might emerge if the bats were all highly stressed by their handling at the beginning of the study, but became habituated as it progressed. However, the bats in the study had been handled regularly before the experiment; additionally, this concern is ruled out by the finding that the samples from the non-focal[23] individuals in the captive colony, which were sampled only at the beginning and end of the experiment, clustered together at each of these time points with the focal bats. Finally, bats in the open colony showed the same foraging patterns during the study (based on Global Positioning System tracking) as they did before and after the study, and they did not leave the colony despite the frequent

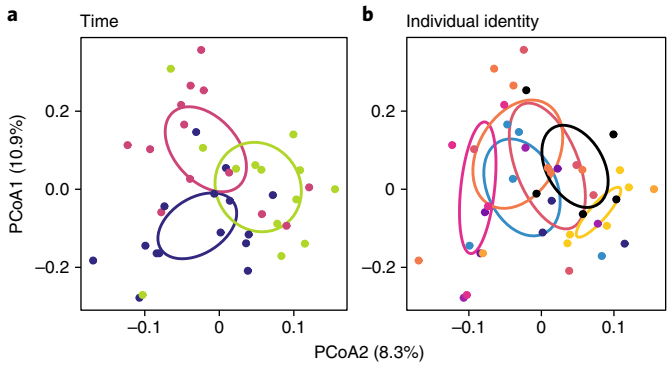

Fig. 5 | Colony and individual-level patterns in profiles of volatile compounds. a,**b**, Samples of the profile of volatile compounds in the fur of the ten focal bats in the captive colony, plotted using PCoA of the Bray–Curtis dissimilarity measure between samples. Each point represents a sample. In **a**, samples are coloured according to the collection date, where dates are divided into the three time trimesters of the 13-week period of the experiment (trimesters 1–3 are coloured blue, red and green, respectively). Only three periods (versus four for the microbiome) were used for volatile analysis due to the smaller number of samples. In **b**, samples are coloured according to the individual from which they were collected. Ellipses represent the areas around the centres of mass (see Methods). Ellipses are not shown for bats from which a small number of samples was collected; however, dots representing the samples from these individuals are presented.

handling. These factors suggest that stress experienced during sampling was not severe.

Recent studies of bats' fur microbiomes, which involved a single snapshot of the fur microbiome of multiple individuals from a number of species[52–54], have found patterns that align well with our findings—namely, that the effect of the local environment (geographic location, specific dwelling site or microhabitat in which the bat was captured) was found to be significant, in some cases above and beyond the species identity and its ecology. This suggests that a bat's fur microbiome may reflect most strongly the specific habitat in which it resides, such as its colony's cave and temporal changes of the microbiome within it, with limited selection being imposed by host factors such as its immune system. The extent to which the immediate environment, such as the bats' cave, determines their fur microbiome, as opposed to the opposite—the bats' microbiome determines the microbiome found in the cave—is unknown. The two are expected to interact, which makes teasing apart these two factors impossible in general, and suggests the study of their interaction as a promising avenue for future research.

Our second main finding is that in the gut microbiome, coordinated change at the colony level is not as prominent, compared with other factors, as in the fur. Why are the dynamics of the fur and gut microbiomes so qualitatively different? One possibility is that the difference is due to the relative roles that common environmental factors play in each of these two modalities: the fur environment is strongly influenced by external factors, while the gut environment is strongly affected by the individual's physiology and immune system, which buffer it from such environmental influences as diet[55,56], which was largely common to all individuals in our colony. This buffering can be seen as adding a 'personalizing' effect, increasing the role of individual identity in determining gut microbiome composition. Another possibility is that the difference is a product of the dynamics of bacterial transmission: the bats' behaviour, which includes frequent and extensive physical contact, has a homogenizing effect on the fur microbiomes—a process from which the gut microbiome is relatively shielded. From the bacterial perspective, one can think of gut bacteria as facing a greater transmission limitation than fur bacteria, creating a structured meta-population in which each individual's gut constitutes an 'island', allowing both neutral and selectively driven divergence between the microbiomes in different guts. These possibilities are not mutually exclusive.

The functioning of the colony's fur microbiome as a single, highly connected biological system might have important implications on the behaviour and ecology of bats and other social species that dwell in close proximity or whose behaviour is otherwise characterized by frequent close physical contact, such as grooming (for example, refs [14,15,19,57,58]). Analysis of the volatiles found on the bats' fur suggests that the fur microbiome may play a role in maintaining the social structure of the colony by facilitating olfactory-based recognition of colony members (see also refs [14,16]). Indeed, analysis of the volatiles in the two colonies (experimental and open) at a single time point revealed that the two differ (see Supplementary Section 5).

Bats' associated microbes have recently received much attention from two specific perspectives. The first views bats as potential reservoirs of zoonotic pathogens that may infect humans[59–63]. The second focuses on the pathogens of the bats themselves, particularly on dynamics of white-nose syndrome[64,65]—a serious emerging epidemic in bats[66,67] (see also refs [53,54]). The highly correlated dynamics of the colony members' fur microbiomes suggests that in bats, and perhaps more broadly in social species that roost in great proximity, the resilience to some types of disease may largely be a colony-level trait, and less a feature of individuals. This has obvious implications, potentially influencing plans for intervention that would mitigate the effects of white-nose syndrome or minimize the prevalence of specific zoonotic pathogens.

At a more theoretical level, our findings allow us to address an ongoing debate regarding the conceptualization of host-microbiome ecology and evolution: some perspectives emphasize the potential utility of a holobiont theory, which regards the host and its associated microbial species together as a meaningful ecological and evolutionary unit[68,69]. Others focus on a metagenomic function-oriented account of the host and its associated microbiota[70–72]. Yet others suggest that no such novel theory is required, and that the ecology of the host and its associated microbiome can best be understood in terms of existing evolutionary and ecological theory (for example, microbe–environment interaction or generalized multi-species Lotka–Volterra dynamics[73]). This debate also recalls an earlier debate in evolutionary biology about levels of selection and ecological dynamics, with different perspectives suggesting the gene, individual, kin group or social group as the meaningful biological unit, whose trajectory in time is most meaningful to track[74,75]. The different perspectives are not mutually exclusive, however, and may contribute complementary insights.

Our findings suggest an additional perspective: that selective pressures on and through the fur microbiome, in species that are characterized by frequent physical contact between individuals, may act mostly at the colony level, and not at the level of the individual, as is commonly assumed. This implies that it may be highly informative to supplement the study of host-microbiome dynamics with a meta-community framework that incorporates hierarchically structured transmission dynamics and in which colonies are the entities whose fate is studied.

Methods

Data collection. Two major colonies of bats reside in the Tel Aviv University zoological garden facility. The first, denoted the captive colony, consisted of 33 bats at the time of this study. The second, denoted the open colony, consisted of ~35 free-ranging bats that could fly out and come back as they wished. From the captive colony, the same ten focal bats (five males and five females) were sampled once a week for their gut and fur microbiome during March to June 2016. Additionally, four focal bats from the open colony were sampled at ten time points for comparison (not all were present at all ten time points, as happens in a free-ranging bat colony; the mean number of samples from each open-colony

bat was seven). The captive colony had been in captivity at the zoological garden of Tel Aviv University for approximately ten years before our study. During these ten years, bats were introduced from the wild periodically to increase genetic variation. The bats were housed within an indoor aviary simulating a natural cave ($2.5 \times 4.0 \times 2.5\,m^3$) with a reversed 12 h day/night cycle. They were fed daily (ad libitum) with a variety of seasonal fruit (see additional details in Supplementary Section 8).

All bats were handled with single-use clean gloves and swabbed for DNA before other measurements were taken, to limit contamination. The samples were taken by sterile culture swab applicators (BD CultureSwab) moistened with Ringer's Solution. Fur sampling was done by sweeping the swab back and forth ten times over each of four different sites: the shoulders, arm pits, stomach and muzzle. Sampling of the gut microbiome was done by holding the bat and squeezing the anus to extract transparent discharge. This discharge was collected by sterile culture swab applicators moistened with Ringer's Solution. *R. aegyptiacus* has a relatively short intestine, not differentiated into small and large parts and with no observed caecum or appendix;[76] the duration of the intestinal pass is approximately 40 min[76,77]. As the bats were sampled after their day fast, and the intestine was free of content, we suggest that this discharge represented the core gut microbiome well without using invasive or lethal techniques (see Supplementary Section 6 for a comparison of the microbiome in these samples with those found in the bats' faeces). All bats were sampled in the same way and in the same order. Additional environmental samples were collected from the fresh food plates, capture nets and air. After sampling, the swabs were sealed in sterile plastic containers, and immediately taken for DNA extraction.

DNA extraction and pyrosequencing. Genomic DNA was extracted from swabs using the PowerSoil DNA isolation Kit (Mo Bio Laboratories), as recommended by the manufacturer. DNA extraction was done close to the date of sampling, and all types of samples collected on the same date (gut, fur, food and controls), from both colonies, were processed together. Extracted DNA samples were stored at −20 °C. PCR amplification of the 16S rRNA gene was carried out with universal prokaryotic primers containing 5′-end common sequences (CS1–341F 5′-ACACTGACGACATGGTTCTACANNNNCCTACGGGAGGCAGCAG and CS2–806R 5′-TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT). A total of 28 PCR cycles (95°C for 15 s, 53°C for 15 s and 72°C for 15 s) were conducted using the PCR mastermix KAPA2G Fast (Kapa Biosystems), and successful amplification was verified by agarose gel electrophoresis. Sample-specific barcodes and Illumina adaptors were added in eight additional PCR cycles, and paired-end deep sequencing of the PCR products from all samples was performed in two sequencing runs on an Illumina MiSeq platform at the Chicago Sequencing Center of the University of Illinois. The sequencing depth ranged from 1,589 to 30,000 sequences per sample. To ensure data evenness, data were rarefied to an equal depth of 1,500 sequences per sample.

Data analysis. Demultiplexed raw sequences were quality filtered (PHRED quality threshold < 20) and merged using PEAR[78]. Sequences shorter than 380 base pairs (after merging and trimming) were discarded. Data were then analysed using the Quantitative Insights Into Microbial Ecology (QIIME version 1.9) package[79] in combination with VSEARCH[80]. Sequences were de-replicated and ordered by size before OTU clustering at the 99% threshold. To reduce spurious formation of OTUs, singleton sequences were not allowed to form new OTUs. Chimeric OTUs were detected and discarded using the UCHIME[81] algorithm against the gold.fa database. Taxonomy was assigned using UCLUST[82] against the QIIME default database (greengenes 13.8).

Analysis downstream from QIIME was done in R and MATLAB. The primary R packages used were Phyloseq, Vegan and Caret. Statistical tests were conducted using their implementation in these packages, with the following settings: PERMANOVA: Adonis{vegan}, permutations = 10,000; Mantel test: mantel{vegan}, method = Pearson, permutations = 10,000; LDA: lda{caret}. In the PERMANOVA tests, no strata were used, and the effect of each variable (for example, date of sampling, individual identity, sex and age) was assessed separately. Note that some of these variables are co-linear. This procedure does not control for pseudoreplication; therefore, a Mantel test was conducted to support assertions regarding significance of variables wherever possible, and PCoA clustering was used for visual demonstrations. PCoA plots were made using ordinate{phyloseq}, with the default settings. The ellipses that describe each group's centre of mass are used for ease of visualization of the centre of the distribution of the points in that group, and reflect the 25% confidence level around the centre of a fit of these points to a multivariate normal distribution. Mantel tests were used to assess whether pairs of samples from the same individual and those from the same date were more or less similar to one another; this was done by performing a Mantel test on the matrix of Jaccard distances and the matrix obtained by assigning 1 to pairs of samples from the same date and 2 to pairs of samples from the same individual (see also ref. [29]).

All results in the main text from Fig. 2 onwards are for the dataset derived from the focal individuals only, following the most conservative procedure of omitting potentially contaminant taxa. This included the removal of all microbial taxa that occurred in the negative controls (blanks) or in more than one of the samples of the bats' food at a frequency above 0.2%. The samples from the food were collected before it was introduced into the colony; thus, any microbial taxa in them were viewed as potential contaminants. This procedure may have omitted taxa that were not contaminants, so the analyses were repeated with the full dataset as well, to confirm that they yielded the same qualitative results. Wherever meaningful, analysis with the full range of samples is included in the Supplementary Material. PERMANOVA tests and LDA analysis were done using the matrix of relative abundances of microbial taxa, and PCoA plots in the main text used Jaccard distances based on the presence or absence of microbial taxa. Analogous analyses with additional distance measures are presented in the Supplementary Material.

Analysis of volatile compounds in fur using gas chromatography. Fur samples were placed in 3 ml vials containing dichloromethane for a minimum of 7 d. The samples were sieved, and extracts were transferred to new insert vials while for each sample the fur was removed, dried and weighed. Two internal standards (udecanal and ergosterol) of known concentration ($0.01\,ng\,\mu l^{-1}$) were added to each extract. Samples were first analysed by combined gas chromatography–mass spectrometry (GC 7890A; MS 5975C; Agilent) using a HP-5MS capillary column that was temperature programmed from 60 to 300 °C at $10\,°C\,min^{-1}$. Compounds were identified by their mass fragmentation and retention times compared with synthetic standards when available. Compound quantification across samples was thereafter performed by gas chromatography with flame ionization detection (CP-3800; Varian) using a DB-1 fused silica capillary column ($30\,m \times 0.25\,mm$ i.d.), temperature programmed as above, using peak integration. We identified 22 peaks in the normalized chromatograms as biological compounds (rather than artificial contaminations) using gas chromatography–mass spectrometry (Supplementary Section 5). After removal of samples that failed to produce data, this process resulted in a matrix of 22 by 41, representing 22 volatiles sampled from 10 individuals over 6 time points (19 samples yielded no peaks, probably because too little fur was collected and thus we had 41 and not 60 samples). Analysis of the resulting dataset was executed, for consistency, using the same methods and scripts as used for the PCoA and PERMANOVA analyses of the microbiome data. Correlations between the abundances of microbial taxa and volatile compounds were carried out at the OTU level. For each of the 22 volatiles, a 41-dimensional vector, representing the levels of this volatile across individuals and times, was created. This vector was then (Pearson) correlated with a 41-dimentional vector representing the levels of an OTU (sampled over the same individuals and dates). Only the 30 OTUs that appeared in at least 50% of the samples of all individuals were used. This procedure was repeated over all 22 volatiles and 30 OTUs, resulting in a (30×22) correlation matrix. Significant correlations were chosen following false discovery rate correction for multiple comparisons.

Ethics. All experiments were performed with permission from the Tel Aviv University Institutional Animal Care and Use Committee (number L-15–031).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data used in this study has been uploaded to SRA at NCBI, and can be found under Bioproject PRJNA494618 (biosample accession numbers: SAMN10226814–SAMN10227267 and SAMN10174956–SAMN10175066).

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Author contributions

O.K., M.W., L.R. and Y.Y. planned the study, analysed the data and wrote the manuscript. M.W., L.R. and L.H. collected and processed the samples. All co-authors commented on the study design, data analysis and manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Research sample	In this paper we report on the temporal dynamics of the fur and the gut microbiome, assessed using 16S rRNA gene amplification, of ten Egyptian fruit bats (Rousettus aegyptiacus) in a captive colony (which constituted 33 individuals; henceforth, the 'captive colony'), and of four individuals from a wild colony (which constituted 20-50 individuals; henceforth, the 'open colony'). The bats in both colonies are habituated to occasional handling, allowing repeated sampling of the same individuals. Each bat was sampled weekly over a period of 13 weeks, in addition to sparser sampling of all 33 individuals in the captive colony.
Sampling strategy	A representative sample of five males and five females from the experimental colony was sampled regularly for three months, yielding the largest dataset of its kind in a non-model organism. In preliminary data from a previous season we conducted a similar analysis and found that a similar sample size is sufficient to reach statistical significance for multiple statistical relations of interest.
Data collection	Two major colonies of bats reside in the Tel Aviv University zoological garden facility. The first, denoted the captive colony, consisted of 33 bats at the time of this study. The second, denoted the open colony, consisted of ~35 free ranging bats that can fly out and come back as they wish. From the captive colony, the same 10 focal bats, 5 males and 5 females, were sampled once a week for their gut and fur microbiome during March to June 2016. Additionally, 4 focal bats from the open colony were sampled at 10 time points for comparison (not all were present at all 10 time points, as happens in a free ranging bat colony; mean number of samples from each open colony bat is 7). The captive colony has been in captivity at the Zoological Garden of Tel-Aviv University for approximately 10 years prior to our study. During these 10 years, bats were introduced from the wild periodically to increase genetic variation. The bats are housed within an indoor aviary simulating a natural cave (2.5x4x2.5 m3) with a reversed 12hr day/night cycle. They are fed daily (ad-lib) with a variety of seasonal fruit (see additional details in Supplementary Section 8). All bats were handled with single use clean gloves and swabbed for DNA before other measurements were taken, in order to limit contamination. The samples were taken by sterile culture swab applicators (BD CultureSwab™) moistened with Ringer's Solution. Fur sampling was done by sweeping the swab, back and forth, 10 times over each of four different sites: shoulders, arm pits, stomach and muzzle. Sampling the gut microbiome was done by holding the bat and squeezing the anus to extract transparent discharge. This discharge was collected by sterile culture swab applicators moistened with Ringer's Solution. Rousettus aegyptiacus has a relatively short intestine, not differentiated into small and large parts and with no observed cecum or appendix (76); the duration of the intestinal pass is approximately 40 minutes (76, 77). As the bats were after their day-fast and the intestine was free of content, we suggest that this discharge well represents the core gut microbiome without using invasive or lethal techniques (see supplementary Section 6 for a comparison of the microbiome in these samples and in those found in the bats' feces). All bats were sampled in the same way and in the same order. Additional environmental samples were collected from the fresh food plates, capture nets, and air. After sampling, the swabs were sealed in a sterile plastic container provided, and immediately taken for DNA extraction.
Timing and spatial scale	The bats were sampled once a week for their gut and fur microbiome during March to June 2016
Data exclusions	No data was excluded apart from candidate phyla suspected to be contamination. The exclusion process for these is described in the methods section.
Reproducibility	The data and code will be made available. The Methods section describes in detail all experimental procedures.
Randomization	NA
Blinding	Samples were processed together and identified only by an ID number.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	*For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.*
Wild animals	Two major colonies of bats reside in the Tel Aviv University zoological garden facility. The first, denoted the captive colony, consisted of 33 bats at the time of this study. The second, denoted the open colony, consisted of ~35 free ranging bats that can fly out and come back as they wish. From the captive colony, the same 10 focal bats, 5 males and 5 females, were sampled once a week for their gut and fur microbiome during March to June 2016. Additionally, 4 focal bats from the open colony were sampled at 10 time points for comparison (not all were present at all 10 time points, as happens in a free ranging bat colony; mean number of samples from each open colony bat is 7). No bats were harmed in the study, and all bats were later further maintained in their respective colonies.
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*