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Diversity and evolution of the primate skin microbiome

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Skin microbes play a role in human body odour, health and disease. Compared with gut microbes, we know little about the changes in the composition of skin microbes in response to evolutionary changes in hosts, or more recent behavioural and cultural changes in humans. No studies have used sequence-based approaches to consider the skin microbe communities of gorillas and chimpanzees, for example. Comparison of the microbial associates of non-human primates with those of humans offers unique insights into both the ancient and modern features of our skin-associated microbes. Here we describe the microbes found on the skin of humans, chimpanzees, gorillas, rhesus macaques and baboons. We focus on the bacterial and archaeal residents in the axilla using high-throughput sequencing of the 16S rRNA gene. We find that human skin microbial communities are unique relative to those of other primates, in terms of both their diversity and their composition. These differences appear to reflect both ancient shifts during millions of years of primate evolution and more recent changes due to modern hygiene.

1. Introduction

The skin is the largest mammalian organ [1,2], and the primary physical barrier between mammals and the outside world. The outermost layer of the skin comprises host cells along with billions of microbial symbionts. Skin microbes inhibit the colonization of opportunistic or pathogenic microbiota [3,4], regulate immune activation [5–7], and produce compounds that function as both pheromones [8,9] and allomones [10]. Skin microbes also influence the attractiveness of hosts to blood-feeding insects, including mosquitoes, known to transmit malaria, dengue and chikungunya [11–14]. The significance of skin microbes to human health and disease suggests that the features of the body that influence its composition might coevolve with host traits. On human skin, the abundance and composition of microbes varies relatively predictably among habitats on the body, as a function of many factors including the distribution of glands and moisture [15–17]. For example, the apocrine glands in the axillary organ in the armpit produce secretions that provide food for the microbes living therein [18–20]. Microbial constituents of the skin microbe communities play an important role in odour creation due to their production of volatile organic compounds [18,19]. Body odour plays a central role in primate society in the

context of mating, child rearing, predatory protection and territorial marking [21,22]. While we are beginning to learn which host and environmental factors influence skin microbes, we know little about how the microbial composition of the skin varies among closely related primates.

The composition of microbes on human skin might be expected to differ significantly from that of our closest relatives, the non-human primates, for at least three reasons. First, even closely related primates differ in the distribution and abundance (and likely chemical composition) of different skin glands. Skin glands provide both food sources and habitat for microbes [23–27]. For example, our closest evolutionary relatives, chimpanzees and bonobos, share the majority of our nuclear genome [28], yet differ in their abundance and location of eccrine and apocrine glands [26,29] (see the electronic supplementary material, text), the form of sebaceous gland exudate [25] and the expression of immune-related genes compared with humans [30]. The non-human apes, in turn, differ from the Old World monkeys (e.g. rhesus macaques and baboons, approx. 30 Myr divergent from the apes) [31] in other attributes of similar features. For example, while humans, chimpanzees and gorillas have apocrine glands clustered in the axillae (armpits), in Old World monkeys apocrine glands are not clustered and can be found (typically at lower densities) covering the entire body and in equal number to eccrine glands [26,29] (electronic supplementary material, text). Second, over the last 100 years, human hygienic behaviour has dramatically changed such that the skin of most humans is now exposed daily to soaps, detergents and underarm products, some of which affect skin microbes [16,32,33]. Third, microbes might differ among primate hosts as a function of their evolutionary dissimilarity [34,35], whether as a result of drift or selection on host traits that influence skin microbes. Collectively, then, we might predict differences in the skin microbiotas among primate hosts, including humans, that reflect both the recent shifts in human hygiene and more ancient divergences in the biology of the skin in addition to host evolutionary history. Specifically, we might predict large differences between the monkey and the ape hosts, and, within the apes, more subtle differences between human and non-human apes.

The skin habitats with high microbial abundance and functional consequences (e.g. on mating or social behaviour) are perhaps the most likely to be strongly influenced by host evolution (akin to the situation in the gut [34]). The axillae have one of the highest bacterial biomasses on the skin surface [36,37] and are directly sustained by food resources primarily provided by apocrine glands [18]. If the influence of evolution is to be apparent on any part of the skin microbiome, it should be in the axillae, especially given the known differences in apocrine gland distribution and density among primates (electronic supplementary material, text).

Here we assess the microbial (bacterial and archaeal) communities in the axillae of five primate species. Samples were collected from humans, zoo populations of chimpanzees, gorillas and baboons, and semi-free-ranging rhesus macaques. We used high-throughput pyrosequencing of the 16S rRNA gene to identify microbes present in primate axillae. We hypothesized that the composition and diversity of axillary microbes would be different for each host species and predicted that their composition would track the phylogenetic relatedness of primate hosts. Exclusive of studies focused on humans, our study is the first to consider the microbes on primate skin with high-throughput sequencing methods. In doing so, we reveal a clear signature of host evolution and biology on the microbiome of the axillae. These results emphasize the role of evolutionary relationships in defining the human skin ecosystem, and have implications for the role of skin microbes in health and disease.

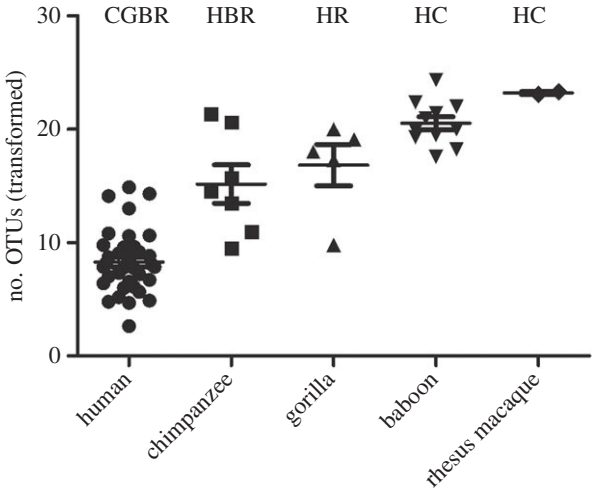

Figure 1. Number of OTUs per primate species after rarefying to 1000 sequence reads per individual and square-root transforming. In order to fit the assumptions of an ANOVA, square-root transformations were conducted because of unequal variances. Levene's Test, one-way ANOVA and Tukey's multiple comparison test were completed on the mean of each host group ($F_{4,62} = 51.3$, $p < 0.05$). Letters above columns correspond to the first letter of the primate host that is significantly different ($p < 0.05$).

2. Results and discussion

(a) Axillary microbial richness

We obtained ribosomal RNA gene sequences from 63 samples of primate axillae using high-throughput pyrosequencing (Roche 454), and processed them using the Quantitative Insights into Microbial Ecology (QIIME) pipeline [38]. To evaluate microbial diversity, QIIME categorizes and groups similar sequences that share a threshold level of sequence identity into a defined operational taxonomic unit (OTU); it then identifies a representative sequence from each OTU group to assign taxonomic lineage. Using a 97% nucleotide-sequence identity threshold to define OTUs, we narrowed our samples to a single axilla sample per individual and rarefied to 1000 sequences per individual (see Methods; electronic supplementary material, figure S1). We identified 5309 unique OTUs from our primate axillae samples; 30 OTUs were unclassified (probably sequencing artefacts), 15 were archaeal in origin and 5264 were bacterial OTUs.

Using the rarefied dataset, we found that humans had the lowest average richness of microbial OTUs per individual (one-way ANOVA and subsequent pairwise testing using Tukey's multiple comparison test: $p < 0.05$; figure 1). Chimpanzees and gorillas hosted a similar richness of skin microbes and each had a significantly lower richness of skin microbial OTUs than did rhesus macaques (one-way ANOVA and Tukey's tests: $p < 0.05$; figure 1). In addition, chimpanzees had significantly higher OTU richness than did baboons (one-way ANOVA and Tukey's tests: $p < 0.05$; figure 1). Furthermore, the axillae of rhesus macaques and baboons had a significantly higher OTU richness than did

those of humans and chimpanzees (one-way ANOVA and Tukey's tests: $p < 0.05$). In general, the higher OTU richness of skin microbes in monkeys relative to apes is in line with expectations if the increased number of axillary apocrine glands in apes [29] tends to favour a subset of bacterial taxa at the expense of diversity (electronic supplementary material, text).

The four most common human skin bacterial phyla—Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes [8]—comprised over 97% of the sequence reads in the human axillae samples (electronic supplementary material, figure S2), similar to that observed in studies focused on human skin microbes [15]. These same phyla made up 85% of the sequence reads in chimpanzees and gorillas, 82% in baboons and 88% in rhesus macaques (electronic supplementary material, figure S2).

(b) Composition of axillary microbes among hosts

To understand the microbial composition among host primates, we first assessed the underlying compositional data matrix (sequence count of each microbial genus by individual host) to confirm that there was structure in the dataset using the KR-means with a Simprof significance test in PRIMER-E v. 7.0.8. We found that there was significant structure in microbial composition among hosts ($R = 0.91$, $p = 0.01$). We then applied a hierarchical approach to assess whether the composition of skin microbes differed predictably among primate groups (monkeys and apes), within each group (monkeys: baboons, rhesus macaques; apes: humans, chimpanzees, gorillas). We then examined the individual variation in skin microbial composition within host species. Finally, we compared the skin microbes of human zoo workers with other humans and to apes living in zoos to explore proximity transfer of microbes. We visualized relative composition per host using non-metric multi-dimensional scaling (NMDS) and employed PerMANOVA (a permutational ANOVA/MANOVA) and PermDISP (permuted dispersion, which tests for homogeneity of dispersions) to statistically evaluate differences among hosts (see Methods).

After demonstrating overall structure by microbial composition of host axillae, we tested primate groups and found that the composition of axillary microbes differed significantly between monkeys and apes (PerMANOVA, $p < 0.01$; figure 2). These differences were primarily driven by *Corynebacterium* and a genus of Staphylococcaceae (not further differentiated using the 16S rRNA segment we sequenced, so hereafter referred to as Staphylococcaceae), both of which were much more abundant in apes than in monkeys; both *Corynebacteria* and Staphylococcaceae/*Staphylococcus* have also been found in high abundance in studies of human skin microbes [15,20,36,39–42]. Together, *Corynebacteria* and Staphylococcaceae contributed to more than 37% of differences among apes and monkeys (SIMPER analysis; table 1). Interestingly, a small number of individual gorillas and chimpanzees had skin microbes that were compositionally more similar to monkeys than was the case for the average gorilla or chimpanzee (figure 2; see circles near triangles). These individual hosts had lower abundances of *Corynebacterium* than other apes (unpaired *t*-test: $p < 0.01$), but no significant difference in Staphylococcaceae (unpaired *t*-test: $p > 0.05$). Despite such differences in microbe composition among individuals within host species, the skin microbes on different host species generally tracked the evolutionary history of those hosts, just as has been shown for gut and saliva microbiomes [35,43] (electronic supplementary material, figure S3*a*,*b*).

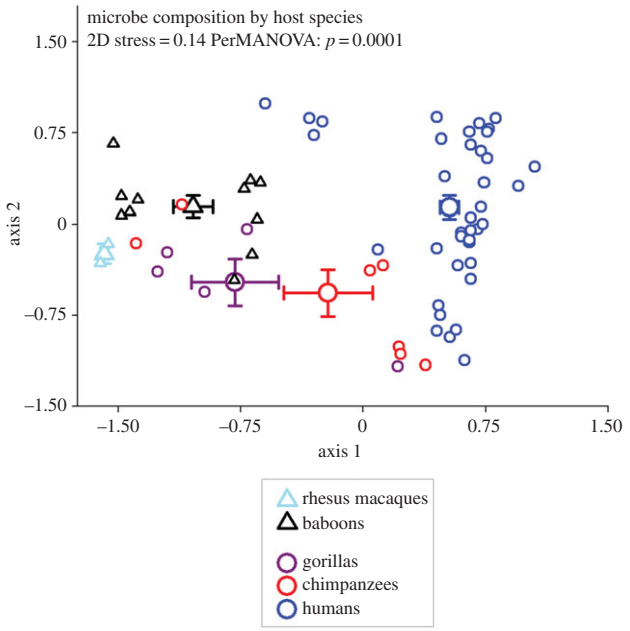

Figure 2. NMDS plot of the composition of skin microbial communities across all five primate species. Each point represents an individual host; triangles represent monkeys (baboons and rhesus macaques), while circles represent apes (gorillas, chimpanzees and humans). Species are coded with different colours. Larger symbols represent the centroid for each species ± 1 se. The composition of skin microbiota on monkeys was significantly different from that of apes (PerMANOVA: $p < 0.01$). Within monkeys, baboons and rhesus macaques had significantly different skin microbiota (PerMANOVA: $p < 0.01$). Within apes, gorillas and chimpanzees had significantly different skin microbiota than humans (PerMANOVA: $p < 0.01$ for both comparisons); however, the composition of gorilla and chimpanzee skin microbiota did not differ significantly from each other (PerMANOVA: $p = 0.12$). (Online version in colour.)

When we investigated differences among individuals within primate groups, we found that within monkeys, there were significant differences between the skin microbiota in the axillae of baboons and in those of rhesus macaques (PerMANOVA, $p = 0.01$; figure 2). *Prevotella* contributed to 10% of the total differences between the two monkey hosts, while Staphylococcaceae was responsible for 8% of the total differences between baboons and rhesus macaques (electronic supplementary material, table S1). *Prevotella*, an anaerobic group of microbes found in the mouth, vagina and gut of humans, represented the most common taxon (22% of all reads; figure 3, green block) in the axillae of rhesus macaques. The abundance of *Prevotella* was greater in the axillae of rhesus macaques compared with baboons (one-way ANOVA: $p < 0.05$; electronic supplementary material, figure S4). The composition of microbes in baboon axillae was diverse. The most common taxon was Staphylococcaceae, which accounted for 12% of reads (figure 3, red block). In contrast to differences in composition within hosts, there were no significant differences in beta diversity (a measure of variability among groups) between baboons (zoo animals) and rhesus macaques (semi-free-ranging; PermDISP: $p = 0.89$; figure 4). This is somewhat surprising given that differences in microbial composition have been previously noted between captive and wild host species [43,44].

Within apes (gorillas, chimpanzees and humans), the axillary microbial communities of humans were significantly

Table 1. Comparison of the average abundances (number of sequence reads) and percentage contributions of the 10 microbial taxa that contributed the most to differences between apes and monkeys (SIMPER analysis, PRIMER-E v. 7.0.8). Undetermined microbial taxa (each of which is a single OTU) are those that did not match sequences for known, named taxa at genus level. In some cases, with longer sequence reads, these taxa (e.g. Staphylococcaceae) might correspond with known phyla, classes, genera and species, respectively.

comparison	family	genus	average abundance		% contribution to differences	% cumulative contribution
			apes	monkeys		
apes versus monkeys	Staphylococcaceae	undetermined	0.37	0.08	20.79	20.79
	Corynebacteriaceae	*Corynebacterium*	0.29	0.04	16.56	37.35
	Prevotellaceae	*Prevotella*	0.02	0.09	4.77	42.11
	Clostridiaceae	*Anaerococcus*	0.06	0.01	3.86	45.97
	undetermined phylum		0.01	0.07	3.61	49.58
	Pasteurellaceae	undetermined	0.00	0.03	1.89	51.47
	Streptococcaceae	*Streptococcus*	0.00	0.03	1.83	53.30
	Ruminococcaceae	*Ruminococcus*	0.01	0.02	1.58	54.88
	undetermined Actinomycetales		0.00	0.03	1.41	56.29
	undetermined Streptophyta		0.01	0.02	1.33	57.62

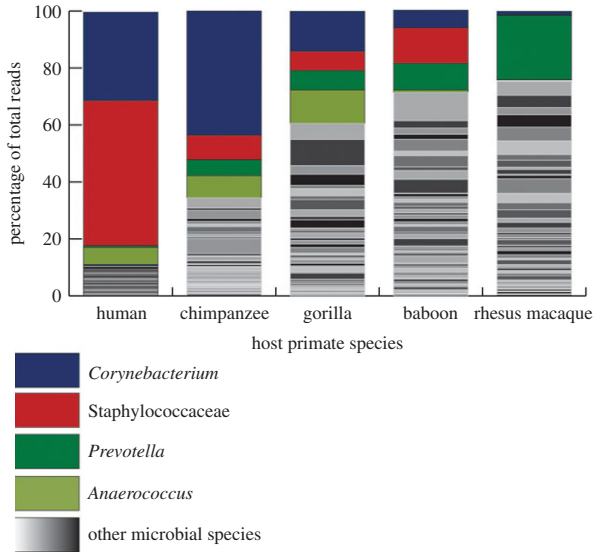

Figure 3. Percentage of total reads of the Staphylococcaceae family, and genera of *Corynebacterium*, *Anaerococcus* and *Prevotella* in each of the five host primate species. Columns represent average sequence reads per individual within host group.

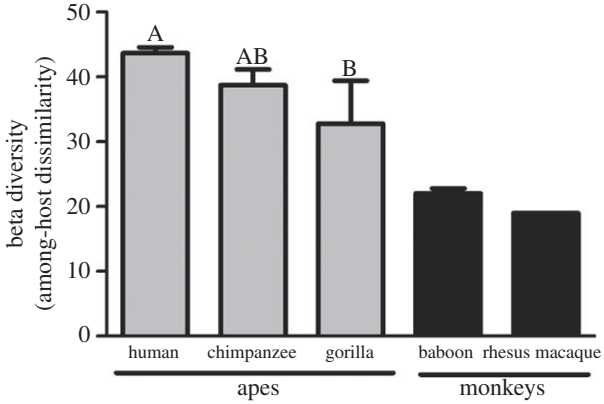

Figure 4. Beta diversity bar charts showing dissimilarity in microbial content (based on number of sequences and taxonomic classification) between primate species within apes and monkeys. Within apes, humans and gorillas were significantly different (PermDISP: $p < 0.05$). There was no significant difference between humans and chimpanzees or chimpanzees and gorillas. Within monkeys, there were no significant differences between baboons and rhesus macaques.

different from those of chimpanzees and gorillas (PerMANOVA: $p < 0.01$ for both). However, there were no significant differences in the composition of skin microbiota between chimpanzees and gorillas (PerMANOVA: $p = 0.12$; figure 2; electronic supplementary material, table S1). Greater abundances of *Corynebacterium* and Staphylococcaceae in humans than in other apes underlie most of this divergence in axillary microbiota between humans and other apes. These two genera contributed 52% of the total compositional differences between humans and chimpanzees, and 41% of the total compositional differences between humans and gorillas (electronic supplementary material, table S1). The abundance of Staphylococcaceae was significantly higher in humans compared with all other primate hosts (one-way ANOVA: $p < 0.05$; electronic supplementary material, figure S4). *Corynebacterium* abundance was significantly higher in humans and chimpanzees when compared with baboons (one-way ANOVA: $p < 0.05$; electronic supplementary material, figure S4).

Humans had the highest level of compositional variability from one individual to the next, in line with the expectation if human underarm products have a strong influence on the axillary microbiome. Recent studies investigating the effects of underarm product use found shifts from *Corynebacterium* to Staphylococcaceae in people who use underarm products such as antiperspirant and deodorant [32,33]. Additionally, people who routinely wore no product hosted higher abundances of *Corynebacterium* than of Staphylococcaceae [32]. Here, our results recontextualize those findings and suggest that in the absence of deodorant and antiperspirant, humans have axillary microbes more similar to apes (more *Corynebacterium*, less

Staphylococcaceae), but that by altering the relative abundance of these taxa, underarm product use makes us less similar to the other apes (electronic supplementary material, table S1). Within host species, the composition of microbes tended to vary more from one individual to the next within ape species versus within monkey species (PermDISP, $p < 0.01$; electronic supplementary material, figure S5).

Given that the skin is constantly in contact with the surrounding environment, and in the light of recent evidence of transfer of microbes during contact sports [45], we investigated whether people who came into contact with non-human primates acquired microbes from the animals they worked with. Individuals who worked with non-human primates did not differ from other humans in terms of their axillary microbiome, nor did they differ from non-human apes (electronic supplementary material, text and figure S6*a*,*b*). Thus, if transfer occurs between humans and non-human animals it does not eclipse the differences intrinsic to host species [43], but it may be associated with more subtle shifts (hence our inability to distinguish those with close contact with other primates compared with none).

(c) A core primate axillary microbiome

Despite the differences among individual hosts and among host species, we identified a core primate axillary microbiome comprising taxa shared by at least 95% of individuals [46]. OTUs of the genus *Corynebacterium* were found in all primate individuals sampled. *Corynebacterium*, *Prevotella*, *Anaerococcus* and a genus of Staphylococcaceae were also among the most abundant taxa based on read number (figure 3; electronic supplementary material, figure S7). These taxa are consistently frequent (found on many individuals) and abundant (present as many reads). Meanwhile, the diversity of OTUs outside of the top four genera increased with evolutionary distance from humans (figure 3; electronic supplementary material, figure S7), an effect likely to be due to a complex mix of the influence of human hygiene, the evolutionary history of the axillary organ and other host differences that have accrued over evolutionary time. Among the most common taxa in the primates most evolutionarily distant from humans (in our sampling), the monkeys, were OTUs of *Prevotella* and Actinomycetales. In addition, the monkeys have a large number of microbial genera (more than 30 genera) in their core axillary microbiome that are generally associated with soil, gut and oral microbiomes such as *Acinetobacter*, Ruminococcaceae and *Porphyromonas*, respectively. It is unclear whether these taxa live on the skin persistently, or represent frequent contamination of the skin with soil and faeces (or both).

3. Conclusion

A large body of recent research has suggested that human exposure to microbes has changed dramatically over the last 100 or 200 years (and prior to that, with the origin of agriculture more than 10 000 years ago), with consequent shifts in gut [35] and oral microbes [43], in extreme cases leading researchers to the 'hygiene hypothesis' [47,48]. Here, we find that the shift is variable among individuals, and rather than being associated with sweeping changes in microbial fauna, it is associated with two key changes. First, humans seem to be less covered with faecal and soil microbes than are other primates, particularly monkeys. Second, humans have a less diverse skin microbial community, more dominated by Staphylococcaceae than is the case for any other primate sampled. The latter is interesting in as much as *Staphylococcus* has long been viewed as the 'normal' skin symbiont. It is also one that has been noted to attract mosquito species, including at least one vector of malaria [11]. Our work suggests the modern composition of our axillary microbiome, including the abundance of Staphylococcaceae, is new at least relative to our divergence with other primates. Whether it is new relative to changes in the last few hundred years remains to be seen. Nonetheless, our findings advance our understanding of the evolutionary context of the diversity of human skin microbiota, and suggest that phylogenetic relatedness among hosts may strongly influence the composition of skin microbiomes across all primates.

4. Methods

(a) Sample collection

Samples from both the left and right human axillae of participants were collected between August 2012 and May 2013. Human volunteers from the North Carolina Museum of Natural Sciences (NCMNS) and North Carolina State University (NCSU) were asked to refrain from using deodorant or antiperspirant for 2 days prior to sampling, but were allowed to shower normally. Participants were told to swab each axilla with a sterile dual-tipped rayon swab (BD BBL Cat no B4320135) for at least 45 s while rotating the swab so that all sides of each swab came in contact with the axillary skin. In this study, we engaged 20 human participants from a concurrent study, in addition to 17 collected specifically for this study, for a total of 37 human participants. We collected samples from non-human primates during routine physicals of baboons, chimpanzees and gorillas at the North Carolina Zoo, and rhesus macaques in Cayo Santiago in Puerto Rico. All zoo primates were captive born except for one chimpanzee born in the wild. None of the gorillas were taking antibiotics at the time of swabbing, but all of the gorillas had been treated within the past six months with a dewormer (mebendazole or pyrantel pamoate). None of the other non-human primates were being given any medication. All zoo primates had outdoor and soil access, and ate vegetarian diets. The rhesus macaques lived entirely outdoors and were rationed with monkey chow. In total, we sampled 11 baboons, seven chimpanzees, five gorillas and two rhesus macaques. Swabs were stored at 4°C until processed. Human samples were processed within weeks of initial sampling; primate samples were processed within months after initial sampling.

(b) DNA extraction and pyrosequencing

Each armpit was swabbed with a dual-tipped rayon swab. One of each of these tips was used for qualitative plating for outreach events at NCMNS; the second was used for DNA extraction for pyrosequencing (454 Roche Genome Sequencer FLX system). DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following manufacturer's protocol with a modification of a two-step elution process into 50 total microlitres of elution buffer C6. DNA was stored at −20° C until amplified and sequenced. Isolated DNA was PCR amplified with 16S rRNA primers (515F and 806R(49)) with 454 adapters and index sequences using Premix Ex Taq (Takara Bio, Siga, Japan) [38]. PCR amplification was done as follows: initial denaturation at 94°C for 2 min, five cycles of 94°C for 20 s, 53°C 30 s, 72°C for 1 min, then 30 cycles of 94°C for 20 s, 55°C for 30 s then 72°C for 1 min followed by a final 72°C extension for 7 min and a 10°C hold. Each host individual had a unique index and PCRs

were run with 3–6 µl of isolated microbial DNA in triplicate in 25 µl reactions with 1.25 µM each primer with a 1X PCR master mix. PCRs were visualized using gel electrophoresis and then triplicate reactions from each individual were pooled and purified using UltraClean-htp 96-well PCR Clean-up kit (MO BIO) per manufacturer's protocol. An equal mass of purified pooled product, as measured by Qubit dsDNA HS Assay Kit (Invitrogen, Eugene, OR, USA), was added to a single tube and then ethanol precipitated to concentrate the mixture. The ethanol-precipitated 16S rRNA pooled mixture was measured by Qubit and was sent for Roche 454 next-generation pyrosequencing (Selah Genomics, Greenville, SC, USA).

(c) Sequence analysis

In addition to the 17 newly collected samples taken from non-human primates and humans for this study, we included data from 20 additional human participants collected for a different study [32]. Combining these two datasets yielded 798 818 sequence reads with 698 799 passing quality and length filters. Amplicons of the V4–V5 region [49] of the 16S rRNA gene were processed and analysed using the QIIME pipeline (MacQIIME 1.7.0-20130523; electronic supplementary material, text) [38]. DNA sequences were processed, filtered and assigned to individuals according to a 13 bp barcode, derived from the standard 12 bp barcode [49] with one bp added *in silico* to all samples in the mapping file and the original fna files in order to combine the Urban *et al*. samples that used the same 12 bp barcodes. Denoising of samples was not done due to flow pattern B processing of 454 sequence data, which enabled longer reads but removed the denoizing capability. OTUs were clustered by Uclust v. 1. 2.22q [40] using a 97% nucleotide similarity threshold such that reads 3% different (or less) from each other were assigned to the same taxon. Taxonomic identities were aligned using PyNAST v. 1.2 and assigned based off Greengenes v. 12-10) [50,51]. OTUs of the family Staphylococcaceae were common in our dataset (more than 50% sequences), but could only be defined at the family-level classification. The OTUs of the family Staphylococcaceae taxon fell within a single 97% de novo OTU, thus we reference them in comparison to other genus-level OTUs. All samples were rarefied to 1000 sequence reads per sample prior to downstream analyses. Five samples were removed from analysis due to limited read depth. Analysing 53 individuals with both left and right samples, there was no significant difference (two-tailed unpaired *t*-test: $p = 0.81$) in genus-level OTU counts in left versus right axilla samples. Because we found no significant difference between left and right axilla samples, and some host individuals provided only one axillary sample, we analysed a single axillary sample for each individual in this study (electronic supplementary material, figure S1). For individuals providing both axillary samples, one side was chosen based on the highest genus-level OTU value by sample per individual. Data from both axillae are deposited in NCBI as Bioproject PRJNA281417 (http://www.ncbi.nlm.nih.gov/bioproject/; electronic supplementary material, text).

(d) Operational taxonomic unit data processing

All further data analysis used only one axillae sample per participant. The homogeneity of variances of host OTUs was assessed using Levene's test (IBM SPSS Statistics for Windows, v. 23.0; Armonk, NY) to determine whether the data fit the assumption of an ANOVA. After square-root transformation, a one-way ANOVA and Tukey's multiple comparison test were used to compare the mean of the square root transformed OTUs in each host group using GraphPad Prism v. 5.01 (La Jolla, CA, USA). Further downstream analysis was calculated using QIIME output at the phylum level (L2) or the genus level (L6). Sequence reads and abundance datasets are available on FigShare under the manuscript title (Figshare.com).

(e) Clustering via KR-clustering

Before conducting statistical analyses of the composition data, we used KR-clustering in Primer v. 7.0.8 [52] to identify whether structure existed with regard to the differences in microbe species composition among individual hosts and host species at the genus level. We constructed a dissimilarity matrix, in which microbial abundances were compared between samples. With this matrix, we conducted the KR-cluster analysis using 100 restarts and a Simprof significance test with two to five groups and $\alpha = 0.001$. Individual hosts clustered more than would be expected based on chance. On the basis of this result, we next analysed the relationship between skin microbial composition and attributes of hosts, which might explain this clustering.

(f) Analysis of skin microbe compositional diversity

We compared the composition of skin microbes among the axillae of individuals of all five primate hosts; we tested whether the differences among host species in the composition of microbial communities were greater than the differences among host individuals within species using a PerMANOVA. All analyses of composition, including Bray–Curtis dissimilarity, non-metric multi-dimensional scaling plot, SIMPER analysis and PermDISP, were conducted using Primer-E v. 7.0.8 with the PerMANOVA ext. v. 1.0.3 [52]. We first constructed a resemblance matrix based on the number of sequence reads of each microbe taxon on each host individual using Bray–Curtis dissimilarities. Bray–Curtis dissimilarity is a standardized metric of compositional dissimilarity among ecological samples, such as swabs of axillae or quadrats in old fields. Values range between 0 and 1, with 0 indicating no difference among samples and 1 indicating that two samples do not share any microbial species [53]. Bray–Curtis dissimilarity has been recommended as a robust measure of ecological distance for complex communities [54]. Next, we created a NMDS with a Type I Kruskal fit scheme and 100 restarts; NMDS is a preferred approach when differences among samples may be due to categorical variables (e.g. host species) rather than continuous gradients (as in detrended correspondence analysis) [55]. To test our *a priori* hypothesis regarding the causes of differences in microbe composition among hosts, we used PerMANOVA with the independent factor of apes (humans, gorillas, chimpanzees) versus monkeys (baboons, macaques; 9999 iterations, and Type III sums of squares). When differences between apes and monkeys were significant, we assessed pairwise differences among host species within apes and within monkeys using PerMANOVA with species as a factor, 9999 iterations and Type III sums of squares. To determine the difference between zoo workers, other humans and zoo animals (chimpanzees, gorillas and baboons), we performed PerMANOVA with the independent factor HumanZoo (human zoo workers + humans working with wild primates, other humans and non-human primates housed in zoos). We performed SIMPER analysis to determine which OTUs contributed the most to differences among host groups.

We assessed the variation in skin microbial composition among host species using PermDISP. Specifically, we transformed the data matrix into a presence/absence matrix, and then constructed a resemblance matrix using Bray–Curtis dissimilarities using these transformed values. Using this matrix, we performed a PermDISP using host cluster as the independent factor, 9999 iterations and centroids as the measure of central tendency. Here, we were testing whether taxa differed in terms of how variable composition was among individuals within the host (e.g. among chimpanzees versus among gorillas). Again, when differences between apes and monkeys were significant, we assessed pairwise differences for each species pair within apes and monkeys as described above.

(g) Core microbiome

Primate core microbe taxa were determined based on the QIIME L6 genus-level output. Microbes found in more than 95% of all individuals sampled across all hosts were determined to be in the primate core microbiome [46]. Microbes found in all non-human primate species were termed non-human primate core microbes. Any microbe found in all monkey individuals (baboons and rhesus macaques) was considered to be a monkey core microbe.

Ethics. All human participants were provided a written informed consent form approved by North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research (IRB) (approval no. 1987) or North Carolina Central Institutional Review Board (approval no. 1201121).

Data accessibility. DNA sequences: NCBI as Bioproject PRJNA281417. Also on FigShare under the manuscript title (Figshare.com).

Authors' contributions. S.E.C. carried out the molecular laboratory work, participated in data and statistical analysis, participated in the design of the study and drafted the manuscript; A.M.S. carried out the statistical analyses and drafted the manuscript; M.E.E. collected field data; J.M.U. helped analyse data; J.H.P.S. and M.L.P. aided in data collection and manuscript revisions; J.E.H. and R.R.D. conceived of the study, designed the study, coordinated the study, participated in data analysis and drafted the manuscript. All authors provided valuable insights on the manuscript and gave final approval for publication.

Competing interests. We have no competing interests.

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