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Fur seal microbiota are shaped by the social and physical environment, show mother-offspring similarities and are associated with host genetic quality

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Abstract

Despite an increasing appreciation of the importance of host-microbe interactions in ecological and evolutionary processes, the factors shaping microbial communities in wild populations remain poorly understood. We therefore exploited a natural experiment provided by two adjacent Antarctic fur seal (*Arctocephalus gazella*) colonies of high and low social density and combined 16S rRNA metabarcoding with microsatellite profiling of mother-offspring pairs to investigate environmental and genetic influences on skin microbial communities. Seal-associated bacterial communities differed profoundly between the two colonies, despite the host populations themselves being genetically undifferentiated. Consistent with the hypothesis that social stress depresses bacterial diversity, we found that microbial alpha diversity was significantly lower in the high-density colony. Seals from one of the colonies that contained a stream also carried a subset of freshwater-associated bacteria, indicative of an influence of the physical environment. Furthermore, mothers and their offspring shared similar microbial communities, in support of the notion that microbes may facilitate mother-offspring recognition. Finally, a significant negative association was found between bacterial diversity and heterozygosity, a measure of host genetic quality. Our study thus uncovers a complex

interplay between environmental and host genetic effects, while also providing empirical support for the leash model of host control, which posits that bacterial communities are driven not only by bottom-up species interactions, but also by top-down host regulation. Taken together, our findings have broad implications for understanding host-microbe interactions as well as prokaryotic diversity in general.

Keywords: skin microbiome, mother–offspring recognition, host control, inbreeding, *Arctocephalus gazella*, pinniped

Introduction

It is increasingly being recognised that most eukaryotic organisms harbour abundant and diverse microbial communities that are intricately linked to all aspects of their host's life cycle. For example, symbiotic microorganisms aid in the acquisition of essential nutrients, influence development and reproduction, educate the immune system and affect social interactions and chemical communication between their hosts (Ezenwa & Williams, 2014; Franasiak & Scott, 2015; Macpherson & Harris, 2004; McFall-Ngai et al., 2013; Scharschmidt et al., 2015; Suzuki, 2017). Consequently, knowledge of host–microbial interactions as well as more generally the factors that shape the microbiota is essential for understanding ecological and evolutionary processes in wild populations. Yet despite decades of research, many of these factors remain elusive and we are only starting to grasp the relative importance of environmental versus host genetic factors (Kurilshikov, Wijmenga, Fu, & Zhernakova, 2017; Rothschild et al., 2018; Snijders et al., 2016).

The development of high-throughput sequencing technologies has facilitated a shift away from small-scale and taxonomically limited culture-based approaches by making it possible to sequence and characterise entire microbial communities from multiple host individuals. This

has stimulated a growing number of studies seeking to uncover patterns of microbial diversity and their underlying drivers. We now know that many attributes of the host's environment have an influence on the diversity and composition of microbial communities. Temperature, for example has been shown to affect the microbiota of a diverse range of host organisms including marine kelp (Minich et al., 2018), sponges (Ramsby, Hoogenboom, Whalan, & Webster, 2018), fruit flies (Moghadam et al., 2018), amphibians (Kohl & Yahn, 2016), and reptiles (Bestion et al., 2017). Furthermore, several recent studies have highlighted the importance of the social environment in shaping an individual's microbiota. More specifically, social group membership has been shown to be an important predictor of microbial community composition in meerkats (Leclaire, Nielsen, & Drea, 2014), hyenas (Theis, Schmidt, & Holekamp, 2012), and many primate species (e.g. chimpanzees, sifaka, baboons; Moeller et al., 2016; Perofsky, Lewis, Abondano, Di Fiore, & Meyers, 2017; Tung et al., 2015) including humans (Song et al., 2013).

Social stress in particular is emerging as an important modulator of the microbiota. Specifically, laboratory rodents subjected to social defeat stress exhibit significantly lower microbial diversity and altered community structures (Bailey et al., 2011; Partrick et al., 2018). The same may also be true of wild populations, as recent studies have shown that oral and gut bacterial diversity correlates negatively with glucocorticoid concentrations in red squirrels (Stothart et al., 2016) and is also influenced by the intensity of social interactions in barn swallows (Levin et al., 2016). However, little else is currently known about the impacts of social stress on bacterial community composition in the wild. For example, high social density, which is associated with increased stress levels in many bird and mammal species (Creel, Dantzer, Goymann, & Rubenstein, 2012) would be expected to reduce bacterial diversity in wild populations but data on this are currently lacking.

It has furthermore been suggested that the microbiota may impact host evolution via the modulation of host social behaviour, especially in the context of chemical communication. The involvement of symbiotic bacteria in olfactory signaling among animals was hypothesized

several decades ago (Albone, Eglinton, Walker, & Ware, 1974; Gorman, Nedwell, & Smith, 1974). Specifically, the fermentation hypothesis of chemical recognition posits that bacteria present in mammalian scent glands metabolise host secretions into odorous compounds, which can be used as olfactory cues for individual recognition between host individuals (Albone et al., 1974; Gorman, 1976). A corollary of this hypothesis is that odour variation among hosts should reflect differences in symbiont community composition. This is supported by studies on meerkats and hyenas (Leclaire, Jacob, Greene, Dubay, & Drea, 2017; Theis et al., 2013) showing that bacterial communities of scent glands are more similar within than among social groups and covary with odour composition. In many mammalian species olfaction also plays a significant role in mother-offspring recognition (Levy & Keller, 2009). Consequently, as microorganisms can be maternally transmitted from females to their progeny (Funkhouser & Bordenstein, 2013), bacterial fermentation might provide a mechanism by which mothers can identify their offspring.

A particularly intriguing aspect of host-associated microbiomes is how hosts control their microbiota. For host species such as mammals that harbor complex symbiont communities, Foster, Schluter, Coyte, and Rakoff-Nahoum (2017) recently proposed the leash model, which posits that the host is under strong selection to evolve mechanisms to keep the microbiota under control, or “on a leash”. Consistent with this, quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) of mice and humans have identified several genetic loci, predominantly involved in immunity and energy metabolism, that are significantly associated with bacterial abundance and community composition (Bonder et al., 2016; Davenport, 2016; Kolde et al., 2018; Kurilshikov et al., 2017; Spor, Koren, & Ley, 2011). Moreover, a growing number of immune-related genes are being linked to a host's ability to limit the opportunistic invasion of host tissues by microbes (Marietta, Rishi, & Taneja, 2015). However, many recent studies have also shown that environmental variation and external

factors such as diet, medication and life style (Lassalle et al., 2018; Rothschild et al., 2018; Shaw et al., 2017; Song et al., 2013) typically explain far more variation in microbial diversity than host genetics.

Studies of wild populations have lagged behind, largely due to the challenges of working with non-model species lacking genomic resources and the difficulty of integrating the confounding influences of environmental variation. So far, it has emerged that gut microbial diversity in sticklebacks can be modulated by host genotype and allelic diversity at immune genes belonging to the major histocompatibility complex (MHC; Bolnick et al., 2014). Mostly unexplored, however, remains the question of how overall host genetic diversity impacts the microbiota. In particular, heterozygosity, a measure of an individual's genetic quality that can be readily quantified using genetic markers, is often negatively associated with individual fitness (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009; Coltman, Pilkington, Smith, & Pemberton, 1999; B. Hansson & Westerberg, 2002; Szulkin, Bierne, & David, 2010) as well as long-term population persistence (Frankham, 2005). Consequently, it is conceivable that the host's potential to control symbiotic microorganisms and to maintain a balanced microbial community could be similarly affected by heterozygosity.

Pinnipeds, a group of marine mammals comprising true seals, sea lions and the walrus, provide an excellent opportunity to study host-associated microbiota due to their unique biology and physiology. As semi-aquatic mammals, pinnipeds are in frequent contact with a large variety of environmental microorganisms originating from both seawater and terrestrial habitats (Bik et al., 2016). Many pinniped species also breed in densely crowded colonies where high levels of social stress and mortality are commonplace (Creel et al., 2012). Furthermore, chemical communication is of fundamental importance to many pinniped species, primarily in the context of kin and mate recognition (Insley, Phillips, & Charrier, 2010; Pitcher, Harcourt, Schaal, & Charrier, 2011; Stoffel et al., 2015), and associations between heterozygosity and various aspects of fitness have been frequently reported (e.g. Acevedo-Whitehouse, Gulland, Greig, &

Amos, 2003; Acevedo-Whitehouse, Petetti, Duignan, & Castinel, 2009; Coltman David, Bowen, & Wright Jonathan, 1998).

A unique natural setup for investigating the effects of environmental and genetic variation on microbial communities is provided by a model pinniped system, the Antarctic fur seal (*Arctocephalus gazella*). At Bird Island, South Georgia, a breeding colony has been intensively studied since the 1980s, with detailed individual-based phenotypic and genetic data having been collected annually since the mid 1990s. These data have revealed heterozygosity-fitness correlations for numerous traits, ranging from early survival and breeding success in females (Forcada & Hoffman, 2014) to body size, attractiveness and lifetime reproductive success in adult males (Hoffman, Boyd, & Amos, 2004; Hoffman, Forcada, Trathan, & Amos, 2007; Hoffman, Hanson, Forcada, Trathan, & Amos, 2010).

Antarctic fur seals breed in colonies that vary in social density by an order of magnitude and rookeries of contrasting density can be found in close geographic proximity, providing a "natural experiment" that controls as far as possible in a natural setting for potentially confounding environmental effects (Meise, von Engelhardt, Forcada, & Hoffman, 2016). On Bird Island, breeding females from two colonies situated just 200m apart (Figure 1) – special study beach (SSB) and freshwater beach (FWB) – are genetically undifferentiated and exposed to the same prevailing climatic conditions (Stoffel et al., 2015). However, SSB has a social density of circa 1.2 females per m^2 whereas the density of breeding females at FWB is only around 0.3 per m^2 (Meise et al., 2016). As a consequence, levels of cortisol and testosterone are chronically elevated at SSB (Meise et al., 2016), which is reflected by a general tendency for trampling and bite injuries to increase with social density in this species (Doidge, Croxall, & Baker, 1984). Animals from these two colonies also differ markedly in their chemical profiles, while regardless of colony, mother-offspring pairs are chemically more similar to one another than expected by chance (Stoffel et al., 2015). As these patterns could potentially be explained by the

fermentation hypothesis, studying fur seal microbiota may also be relevant to understanding chemical communication.

Here, we collected skin swabs and genetic samples from 48 fur seal mother-offspring pairs from SSB and FWB and used 16S amplicon sequencing to characterise their bacterial communities. We hypothesised (i) that bacterial diversity should be lower at the high density colony (SSB) due to the suppressive effects of elevated social stress on microbial communities; and (ii) that mothers and their pups should carry similar microbiota, reflecting their chemical similarity (Stoffel et al., 2015). We additionally genotyped all of the individuals at 50 hypervariable microsatellite loci and regressed multilocus heterozygosity against microbial diversity. According to the leash model of host control, we would expect to find a negative association between individual heterozygosity and overall bacterial diversity as higher quality individuals should be more effective at suppressing non-beneficial microbes.

Material and Methods

Study site and sample collection

Samples were collected from 48 *A. gazella* mother-offspring pairs (total *n* = 96 individuals) during the 2014/2015 breeding season from the two breeding colonies on Bird Island, South Georgia (54° 00 S, 38° 02 W; see Figure 1; and Table S1, Supplementary Information for details). Breeding females and their pups were captured and restrained on land as part of annual routine procedures of the Long Term Monitoring and Survey program of the British Antarctic Survey (BAS) following standard procedures (Gentry & Holt, 1982). A small skin sample was collected from each individual for genetic analysis using piglet ear notching pliers and these were stored individually at -20 °C in the preservative buffer 20% DMSO saturated with salt.

Microbial samples were obtained by rubbing a cotton swab across the animal's cheek, underneath the eye and behind the snout. Each swab was individually preserved in a collection tube containing liquid Amies medium and stored at -20°C. Many studies attempt to eliminate contamination by environmental bacteria by collecting environmental controls and subsequently removing sequences found in these controls from analyses (e.g. Apprill et al., 2017). However, because fur seals are in constant contact with the substrate, we assume that most if not all bacteria will likely be common to both skin and environmental samples, although data are lacking with which to test this. We therefore considered transient environmental bacteria as part of the overall skin microbiota and did not exclude them from the current study.

All of the fieldwork procedures were approved by the BAS Ethics Review Committee and samples were collected and held under permits from the Department for Environment, Food and Rural Affairs (DEFRA), and in compliance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Bacterial DNA extraction and amplicon sequencing

Bacterial DNA was extracted from the Amies media using the BiOstic Bacteremia DNA Isolation Kit (Mo Bio Laboratories, Inc.). Briefly, the tubes containing the swab and Amies medium were centrifuged at low speed to collect all of the liquid in the bottom of the tube (~800 μL). Visible pellets were resuspended by carefully pipetting up and down several times. The liquid was then transferred to new 2 mL collection tubes and all subsequent steps were performed according to the manufacturer's instructions. A detailed description of the protocol is provided in the Supplementary Information.

Amplicon sequencing libraries were prepared for the Illumina MiSeq System following the Illumina 16S Metagenomics Sequencing Library Preparation guideline document. We used the primers V3-V4-341F (5'-CCTACGGGNGGCWGCAG-3') and V3-V4-805R (5'-

GACTACHVGGGTATCTAATCC-3') to amplify a ~550 bp fragment of the V3–V4 region of the 16S rRNA gene. A detailed description of all of the library preparation steps can be found in the Supplementary Information. The pooled libraries were sent to the Cebitec sequencing facility in Bielefeld, Germany, where they were 300bp paired-end sequenced on a single MiSeq lane using the MiSeq Reagent Kit v3 (lllumina, Inc., San Diego, CA, USA).

Microsatellite genotyping and genetic analysis

Genomic DNA was extracted from each sample using a standard phenol-chloroform protocol (Sambrook, Fritsch, & Maniatis, 1989) and genotyped at 50 polymorphic microsatellite loci as described by Stoffel et al. (2015). The loci were PCR amplified in eight separate multiplexed reactions using a Type It Kit (Qiagen). The following PCR profile was used: one cycle of 5 min at 94 °C; 24 cycles of 30 s at 94 °C, 90 s at T_a °C and 30 s at 72 °C; and one final cycle of 15 min at 72 °C (see Table S2 for details of the loci together with mastermix-specific annealing temperatures, T_a). Fluorescently labeled PCR products were subsequently resolved by electrophoresis on an ABI 3730xl capillary sequencer (Applied Biosystems, Waltham, MA, USA) and allele sizes were scored automatically using GeneMarker v. 1.95 (SoftGenetics, LLC., State College, PA, USA) with additional manual inspection and where necessary correction of the traces to ensure high genotype quality.

All loci were tested for linkage disequilibrium (LD) and deviation from Hardy-Weinberg equilibrium (HWE) running Genepop on the Web (Raymond & Rousset, 1995) with 1,000/10,000 dememorizations (LD/HWE), 100/1,000 batches and 1,000/10,000 iterations per batch. False discovery rate (FDR) corrections (Benjamini & Hochberg, 1995) with an alpha level of 0.05 were applied to the resulting *p*-values to account for multiple testing. These analyses were conducted separately for mothers and pups.

Allosuckling in Antarctic fur seals can lead to incorrect assignments of mother-offspring pairs in the field (Hoffman & Amos, 2005). We therefore tested whether our putative mother-offspring pairs were genuine, both by calculating for each pair the probability of maternity in Colony v.2.0.6.4 (Jones & Wang, 2010) and by quantifying pairwise relatedness values (Wang, 2002) using the R package related v1.0 (Pew, Muir, Wang, & Frasier, 2015). A single pup (pup P22) was excluded from these analyses as it failed to genotype at 47 microsatellite loci. Both analyses indicated that five of the mother-offspring pairs identified at SSB (Pair11, 13, 15, 46, and 49) were unrelated, consistent with a previous genetic study (Hoffman & Amos, 2005) and indicative of widespread allosuckling. All of these pairs were subsequently either removed from analyses that required pair information or recoded as unrelated in the remaining analyses.

OTU generation pipeline

Demultiplexed 300 bp paired-end reads were provided by the sequencing facility. The quality of the raw data was assessed using FastQC v. 0.7.2 (Andrews, 2010). For read processing, we followed the guidelines of the UPARSE OTU/denoising pipeline (Edgar, 2013) implemented in USEARCH v. 9.2.64 (R. C. Edgar, 2010). Briefly, after merging forward and reverse reads, primers were trimmed using Cutadapt v. 1.9.1 (Martin, 2011). Low quality sequences were then filtered out of the dataset with an expected error threshold of 1.0 (Edgar & Flyvbjerg, 2015). The remaining high quality sequences were de-replicated to retain only unique sequences, which were then clustered into 97% operational taxonomic units (OTUs). OTUs were classified using the sintax command (Edgar, 2016) with the RDP training set containing species names provided on the USEARCH manual pages and a confidence cutoff of 0.8. OTUs with low confidence classifications at the domain and phylum level, together with chloroplast, archaeal or mitochondrial assignments were discarded after manual inspection. Finally, an OTU table was generated for the final set of OTUs by mapping the merged and trimmed raw reads of each sample against the OTUs with a 97% identity threshold. Following Bokulich et al. (2013), OTUs

that were represented by less than 0.005% of all reads were trimmed from the table. To test if uneven sequencing depth across samples might impact the calculation of diversity estimates, we generated an additional OTU table containing rarefied counts using the single_rarefaction.py script in QIIME v1.9.1 (Caporaso, Kuczynski, et al., 2010). Counts were rarefied to 10,000 reads per individual after excluding samples from two pups (P24, P39) with less than the required read count. A detailed description of all steps, as well as scripts for processing the data and summary statistics obtained after each step of the pipeline are provided in the Supplementary Information text, and Datasets S1, and S2.

Composition of the skin microbiota

We characterised the overall composition of the *A. gazella* skin microbiota, as well as the core microbiota, defined as OTUs present in at least 90% of the samples, by summing up read counts at different taxonomic levels (e.g. phylum, genus, OTU) over all of the 96 sampled individuals using the non-normalised OTU table. These and all subsequent analyses were conducted in R (Team, 2016) using the graphics tools of the package Phyloseq v. 1.22.3 (McMurdie & Holmes, 2013). All of the code used for our analyses is provided as an R markdown document (Supplementary Information Dataset S3).

Alpha diversity

To quantify bacterial diversity within each sample (i.e. alpha diversity), we calculated the Jost index of order 1 in USEARCH, which is the effective number of species (Jost, 2006) given by the Shannon index (Chao, Chiu, & Jost, 2010) (see Supplementary Information for more information). Alpha diversity estimates calculated from the non-normalised and rarefied OTU tables were highly correlated ($r = 0.99$, $p < 0.001$; Figure S1) thus, in order to retain all of the individuals in our analyses, we focused on estimates based on non-normalised counts. To

investigate whether alpha diversity differs between the two breeding colonies as well as between the two age cohorts (adults and pups), we fitted a linear mixed model (LMM) with square root transformed alpha diversity as response variable, and breeding colony (FWB, SSB) and age (mother, pup) as fixed effects. This analysis was performed using the lme4 package v. 1.1-15 (Bates, Maechler, Bolker, & Walker, 2015) in R. Mother-pup pair ID was included in the model as a random effect to account for the non-independence of the two alpha diversity estimates of a pair. Pairs that were identified as unrelated by parentage analysis were assigned different pair IDs to allow the individuals in question to be retained in the analysis. Likelihood ratio tests were performed using the anova function to determine significance of the predictor variables. We calculated the variance explained by the fixed effects as well as the variance explained by both, the fixed and random effects (marginal and conditional coefficients of determination, $R^2_{(m)}$ and $R^2_{(c)}$, respectively) (Nakagawa & Schielzeth, 2013) using the r.squaredGLMM function from the MuMIn package v. 1.40.0 (Bartoń, 2017). We repeated the analysis with alpha diversity estimates calculated from OTUs belonging to the four dominant phyla only (Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria; 613 OTUs) to gauge the robustness of the pattern. We further tested for an effect of sex on bacterial diversity by fitting a linear model (LM) with square root transformed alpha diversity as response variable, and breeding colony (FWB, SSB) and sex (female, male) as fixed effects using the pup subset of the data.

Beta diversity

To investigate differences in bacterial composition among samples (i.e. beta diversity) we calculated Bray-Curtis and weighted UniFrac distance matrices. We normalised the raw counts using cumulative sum scaling (CSS) (Paulson, Stine, Bravo, & Pop, 2013) implemented in the R package metagenomeSeq v. 1.14.2 (Paulson, Talukder, Pop, & Bravo) to account for uneven sequencing depth. Following Thorsen et al. (2016), we additionally Log(x + 0.0001)-

transformed the data and corrected the transformed values by subtracting the log of the pseudocount to preserve zeros from the original counts. UniFrac distances require knowledge of the phylogenetic relationships among the OTUs. We therefore used the QIIME implementations of PyNAST (Caporaso, Bittinger, et al., 2010) and FastTree (Price, Dehal, & Arkin, 2009) to align all of the bacterial sequences and construct a phylogenetic tree, which was rooted using an archaeal sequence (GenBank accession AAU11044) as outgroup. Prior to distance calculations, this outgroup was trimmed from the tree using the ape package v. 4.1 (Paradis, Claude, & Strimmer, 2004). Beta diversity was visualised through principal coordinate analysis (PCoA). We tested for differences in beta diversity between *a priori* defined groupings (i.e. breeding colonies, age groups, sexes (pups only), and mother-pup pairs) by performing non-parametric analysis if similarities (ANOSIM) (Clark, 1999) on Bray-Curtis dissimilarity matrices with 10,000 permutations in the R vegan package v. 2.4-6 (Dixon, 2003). ANOSIM of mother-pup pairs was performed separately both including and excluding the five unrelated pairs.

To investigate whether similarity in bacterial community composition between individuals could be driven by genetic relatedness or spatial proximity, we performed Mantel tests on dissimilarity matrices using the vegan package. We tested for associations between pairwise relatedness (based on 50 microsatellite loci) and Bray-Curtis dissimilarity using Spearman rank correlations with 1,000 permutations of the Bray-Curtis matrix to assess significance. Information on spatial proximity was available for SSB only, where pupping locations have been recorded to the nearest square meter. We calculated a matrix of Euclidean distances between pupping coordinates and tested for a correlation with Bray-Curtis dissimilarity using Spearman rank correlations with 1,000 permutations of the Bray-Curtis matrix to assess significance.

Differential abundance analysis

To identify those OTUs that are the main drivers for the observed alpha and beta diversity patterns, we tested for differential abundance (DA) by performing a negative binomial Wald test implemented in the DESeq2 v. 1.12.4 extension (Love, Huber, & Anders, 2014) of the Phyloseq package. Only OTUs with a significance level (alpha) below 0.01 after FDR corrections were considered. Groups tested were: FWB against SSB, mothers against pups, FWB mothers against FWB pups, SSB mothers against SSB pups, FWB mothers against SSB mothers, and FWB pups against SSB pups. In order to visualise overall differences in OTU abundance across samples, we also constructed a heatmap of the Log(x + 0.0001)-transformed CSS normalised OTU counts from the beta diversity analysis using complete linkage hierarchical clustering of Euclidean distances as implemented in the pheatmap package v. 1.0.8 (Kolde, 2015).

Functional analysis of OTUs

We performed functional analysis of the Antarctic fur seal skin microbiome using the PICRUSt software package (Langille et al., 2013). As PICRUSt can only predict metagenomic function from closely related genomes and is not a true representation of metagenomic content, we present the procedures and results of this analysis in the Supplementary Information.

Associations between heterozygosity and bacterial diversity

To investigate the effect of host genetic quality on microbial communities, we used inbreedR v. 0.3.2 (Stoffel et al., 2016) to calculate each individual's standardised multilocus heterozygosity (sMLH), defined as the total number of heterozygous loci in an individual divided by the sum of the average observed heterozygosities in the population over the subset of loci successfully typed in that individual (Coltman et al., 1999). We examined the relationship between an individual's heterozygosity and bacterial alpha diversity by fitting a LMM with alpha diversity as the response variable and sMLH, breeding colony and age as fixed effects. We also included interactions between sMLH and breeding colony, and sMLH and age, to test whether the effect

of sMLH on alpha diversity differed by colony or age class respectively. As above, mother-pup pair ID was included as a random effect to account for non-independence and pairs that were identified as unrelated by the parentage analysis were assigned different pair IDs. Alpha diversity was square root transformed to achieve normality and heterozygosity was centered around the mean ($\bar{x} = 1.0$). Model estimates were calculated from a LMM that was refitted without the non-significant interaction terms and statistical significance of the predictor variables was determined through likelihood ratio tests using the anova function. To quantify the amount of variance explained, we calculated $R^2_{(m)}$ and $R^2_{(c)}$ as described above. To test if uneven sequencing depth across samples might impact the model estimates, we generated 100 additional OTU tables rarefied to 10,000 reads using the multiple_rarefactions_even_depth.py script in QIIME and calculated alpha diversity from each table (two samples with less than the required read count were excluded from the analysis). Additional LMMs were fitted for all alpha diversity tables as described above and the resulting estimates were compared to the original results.

To investigate whether the relationship between heterozygosity and alpha diversity could be due to inbreeding or local effects involving one or a small number of specific loci (Hansson & Westerberg, 2008), we first calculated the two-locus heterozygosity disequilibrium estimator, g_2, which provides a measure of the extent to which heterozygosity is correlated across loci (David, Pujol, Viard, Castella, & Goudet, 2007) in inbreedR with 10,000 permutations and 10,000 bootstraps. Sensitivity of g_2 to the number of loci was evaluated by randomly selecting different sized subsets of loci and recalculating g_2 1,000 times. We also tested for the possible involvement of local effects using the approach of Szulkin et al. (2010). Specifically, using an F-ratio test, we compared a model of alpha diversity containing multilocus heterozygosity (MLH– the sum of all single locus heterozygosities over all loci; note that MLH is not the same as sMLH) with a model in which MLH was replaced by separate terms for the heterozygosity of each of the 50 microsatellite loci. Local effects can be identified if the second model explains significantly

more variance than the first model. For this analysis, we imputed missing heterozygosity values at individual loci as described by Szulkin et al. (2010).

Results

Composition of the Antarctic fur seal skin microbiota

We sequenced the 16S rRNA V3–V4 region from the skin microbial communities of 48 Antarctic fur seal mother-pup pairs from two breeding colonies (Figure 1) generating 6,038,381 read pairs that were processed and clustered into 788 OTUs. The OTUs were represented by a total of 3,173,550 mapped sequences (merged paired-end reads) with an average depth of 33,058 sequences per sample (range = 3,505 to 83,364). A total of 20 bacterial phyla were identified, with Proteobacteria (38.8%), Bacteroidetes (21.9%), Firmicutes (21.3%) and Actinobacteria (11.4%) dominating the assemblage (Figure 2; Table S3). The most common bacterial genera were *Psychrobacter* (27%), *Chryseobacterium* (6.5%), and *Jeotgalibaca* (2.9%) but over a quarter of the sequences (27.4%) were mapped to OTUs of unknown genus classification due to our conservative choice of reference database. The core microbiota comprised 123 OTUs that were present in at least 90% of the samples, of which 29 OTUs were found in all of the individuals (Table 1; Table S4).

Microbial community structure in relation to breeding colony, age class and sex

Bacterial diversity and community composition were both strongly influenced by breeding locality but not by age class or sex. Specifically, alpha diversity differed significantly between the two breeding colonies, with higher diversity being found at FWB (β = -1.64 ± 0.43 SE; LRT: χ^2 (1)= 13.19, $p < 0.001$; Figure 3; Table S5). By contrast, no significant differences were found between age classes (β = -0.17 ± 0.34 SE; LRT: χ^2 (1) = 0.24, $p = 0.625$; Figure 3; Table S5) or between male and female pups (β = -1.02 ± 0.60 SE; t = -1.70; $p < 0.096$; Figure S2, Table S6).

Very similar results were obtained when analysing only the subset of OTUs belonging to the four dominant phyla (Figure S3, Table S5). Similarly, beta diversity analyses revealed that bacterial communities were significantly more similar within than between the two breeding colonies, both overall (ANOSIM, global $R = 0.877$, $p < 0.001$; Figure S4) and separately for mothers (ANOSIM, global $R = 0.946$, $p < 0.001$) and offspring (ANOSIM, global $R = 0.808$, $p < 0.001$). This is reflected in the results of the principal coordinate analysis, which clustered individuals from the two colonies separately (Figure 4A). No significant differences in microbial community composition were found between the age classes (ANOSIM, global $R = 0.006$, $p = 0.217$, Figure 4A; Figure S4) or male and female pups (ANOSIM, global $R = -0.007$, $p = 0.501$). However, when the colonies were analysed separately, significant differences were observed between age classes (FWB ANOSIM, global $R = 0.084$, $p < 0.01$; SSB ANOSIM, global $R = 0.081$, $p < 0.01$) but not between male and female pups (FWB ANOSIM, global $R = 0.117$, $p = 0.1$; SSB ANOSIM, global $R = -0.042$, $p = 0.72$).

Microbial similarity between mothers and their pups

Mother-offspring pairs were significantly more similar to each other in their bacterial communities than expected by chance (Figure 4B) regardless of whether the data were analysed together (ANOSIM, global $R = 0.602$, $p < 0.001$; Figure S4) or separately for each colony (FWB ANOSIM, global $R = 0.349$, $p < 0.001$; SSB ANOSIM, global R 0.456, $p < 0.001$). Furthermore, including the five mother-offspring pairs that according to the parentage analysis were unrelated in the analysis did not have a strong effect on the results (ANOSIM, global $R = 0.615$, $p < 0.001$; SSB ANOSIM, global $R = 0.408$, $p < 0.001$). With the exception of a single pair, the non-filial pups clustered closely together with their assigned mothers (Figure 4B), suggesting that microbes might be shared due to factors other than genetic relatedness.

Microbial similarity, host relatedness and spatial proximity

No association was found between microbial similarity and genetic relatedness, either overall (Mantel's r = 0.017, p = 0.21) or when the data were analysed separately for mothers (Mantel's r = 0.032, p = 0.18) and pups (Mantel's r = 0.039, p = 0.17). Similarly, there was no significant relationship between beta diversity and the spatial proximity of either mothers (Mantel's r = 0.003, p = 0.49) or pups (Mantel's r = -0.010, p = 0.52) at SSB.

Patterns of differential abundance

In order to identify the bacterial taxa responsible for observed differences in microbial community composition between the breeding colonies as well as between mothers and pups, we performed differential abundance (DA) analysis. The majority of OTUs (n = 655, 83.1%) differed significantly in their abundance between the colonies (Figure 5; n = 579, 73.5% when comparing only mothers and n = 462, 58.6% when comparing only pups, Figure S5), suggesting that colony-level differences are broad-sweeping rather than being driven by a handful of dominant bacterial taxa. This pattern is clearly discernible by eye in the heatmap shown in Figure S6, which also gives an impression of the magnitude of DA between bacterial assemblages from the two breeding colonies. The bacterial phyla that were most strongly over-represented at FWB were Acidobacteria, Candidatus Saccharibacteria, Verrucomicrobia, Planctomycetes, Cyanobacteria, Chloroflexi, and Gemmatimonadetes (Figure 5). By contrast, a single bacterial phylum, Fusobacteria, was over-represented at SSB (Figure 5; Table S7). Additionally, differences in the bacterial community composition of mothers and pups revealed by ANOSIM analyses within each breeding colony were supported by the finding of 155 significantly differentially abundant OTUs between the two age classes (77 at FWB and 63 at SSB; Figure S5). At FWB, similar numbers of OTUs were over-represented in both age classes (37 in mothers and 40 in pups), whereas at SSB the majority of OTUs were over-represented in mothers (55 compared to 8 in pups).

Relationship between host multilocus heterozygosity and bacterial diversity

Individual multilocus standardised heterozygosity (sMLH) based on 50 microsatellite loci was significantly associated with bacterial alpha diversity (β = -6.06 ± 2.43 SE; LRT: χ^2 (1) = 5.69, p = 0.017) after controlling for both breeding colony (β = -1.68 ± 0.40 SE; LRT: χ^2 (1) = 15.62, p < 0.001) and age class (β = -0.29 ± 0.37 SE; LRT: χ^2 (1) = 0.64, p = 0.425). The direction of the relationship was negative, indicating that heterozygous animals carry less diverse microbial communities (Figure 6, Table S8). The slope of the relationship did not differ significantly between the breeding colonies (sMLH*colony interaction; β = 7.75 ± 4.91 SE; LRT: χ^2 (1) = 2.62, p = 0.106; Table S9) or by age class (sMLH*age interaction; β = -0.32 ± 4.99 SE; LRT: χ^2 (1) = 0.01, p = 0.943; Table S9). Almost identical model estimates were recovered when alpha diversity was calculated from multiple rarefied tables (Figure S7), indicating that the model is robust to variation in sequencing depth.

Finally, the two-locus heterozygosity disequilibrium estimator, g_2, was positive but did not differ significantly from zero for our dataset (g_2 = 0.0011 ± 0.0013 S.E., p = 0.12; Figure S8). However, a statistical model containing separate terms for the heterozygosity of each of the loci did not explain significantly more variance than the model fitting only multilocus heterozygosity (MLH, $F_{93,44}$ = 0.956, p = 0.563). This suggests that the association between sMLH and alpha diversity is unlikely to be driven by one or a small number of loci. Consequently, our results are consistent with an effect of host inbreeding on alpha diversity.

Discussion

We used 16S rRNA amplicon sequencing to investigate how environmental and genetic sources of variation shape host-associated microbial communities in a wild vertebrate. At the population level, we found profound differences in skin bacterial community composition that are consistent with variation in social density as well as with the presence of a freshwater

stream in one of the fur seal colonies. At the individual level, mothers and their pups were found to share similar microbial communities, in line with a potential role of bacterial fermentation in kin recognition in marine mammals. Finally, we also uncovered a negative association between host heterozygosity and bacterial diversity that is indicative of an effect of host genetic quality on microbial communities.

Antarctic fur seal skin microbiota

Several recent studies have characterised microbial diversity from the gut and faeces of pinnipeds (Delport, Power, Harcourt, Webster, & Tetu, 2016; Nelson, Rogers, Carlini, & Brown, 2013). By contrast, we specifically focused on the skin microbiota for two reasons. First, a previous study of Antarctic fur seal mother-offspring pairs from the same locations uncovered colony-specific chemical signatures as well as chemical similarities between mothers and their offspring (Stoffel et al., 2015), suggesting that olfactory cues could be important in mother-offspring recognition (Pitcher, Charrier, & Harcourt, 2015). Consequently, we wanted to test whether bacterial fermentation mediated by skin microbes could play a role in generating these patterns. Second, as skin swabs are easy to collect and minimally invasive, focusing on the epidermal microbiota enabled us to collect samples from a large enough number of individuals not only to look for population-level differences, but also to investigate the genetic underpinnings of bacterial diversity at the individual level.

We have shown that bacterial communities collected from the skin of Antarctic fur seals are dominated by the four phyla Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria, which is broadly in line with patterns found in many terrestrial vertebrates (Avena et al., 2016). By contrast, the comparatively high abundance of Bacteriodetes might be more typical of marine mammals, as Bacteriodetes are also abundant on humpback whale and orca skin (Apprill et al., 2014; Hooper et al., 2018). Congruent with a strong environmental influence, we also documented a high relative abundance of *Psychrobacter*, a salt-tolerant bacterial genus that

has been described from many extreme, particularly cold environments including sea ice, Antarctic soil and the deep sea (Bozal, Montes, Tudela, & Guinea, 2003) as well as from humpback whale skin (Apprill et al., 2014). Consequently, the microbial communities that we characterised in this study are exactly what would be expected for an Antarctic marine mammal. Knowledge of the core microbiota of this species might further provide a useful tool for future population monitoring, as core members of the microbial community likely provide critical services to their hosts (Hernandez-Agreda, Gates, & Ainsworth, 2017) and changes in these communities could point to a decline in overall population health.

Differences in microbial communities between colonies

Several previous studies have reported population-specific differences in microbial community composition among taxonomically diverse host species (e.g. Delport et al., 2016; Smith, Snowberg, Gregory Caporaso, Knight, & Bolnick, 2015). However, a tendency of many studies to focus on relatively large geographic scales, from tens to thousands of kilometres (although see Bates et al., 2018; Jani, Knapp & Briggs 2017) has made it difficult to disentangle the contributions of host genetics and environmental variation (Smith et al., 2015). This is because geographically distant populations tend to differ both genetically and ecologically, as isolation-by-distance and isolation-by-environment are often confounded (Shafer & Wolf, 2013). Our study design controlled for this as far as possible in a natural setting, as the two sampled colonies are immediately adjacent to one another and therefore experience the same prevailing climatic conditions. Moreover, both colonies consist of a more or less identical homogenous cobblestone substrate, while seals occupying these two localities are not genetically differentiated (Stoffel et al., 2015) allowing us to exclude the possibility that differences in host-associated microbial communities could be associated with population structure. Thus, ostensibly the colonies differ in just two main respects – SSB has a higher social density that is reflected by elevated stress hormone levels (Meise et al., 2016) and FWB also contains a small stream, which could be a source of freshwater-associated bacteria.

We found striking differences in the bacterial community composition of the two colonies, with alpha diversity being significantly lower in animals breeding in the high density colony, regardless of both age class and sex. This is at odds with previous studies of pikas and swallows that found positive relationships between gut microbial diversity and both social density (Li et al., 2016) and the frequency of body contact interactions (Levin et al., 2016). However, stressful conditions and concomitantly high glucocorticoid concentrations are known to reduce microbial diversity in rodents, birds and fishes (Bailey et al., 2011; Noguera, Aira, Perez-Losada, Dominguez, & Velando, 2018; Partrick et al., 2018; Stothart et al., 2016; Zha, Eiler, Johansson, & Svanback, 2018). Consequently, as cortisol levels are over four times higher at SSB than FWB (Meise et al., 2016), reduced microbial diversity at SSB could be suggestive of a negative impact of social density on bacterial communities mediated by the suppressive effects of socially induced stress.

In line with this hypothesis, stress-induced decreases in protective symbionts along with host immunosuppression have been suggested to lead to the proliferation of pathogenic taxa and render the host more vulnerable to infections (Alverdy & Luo, 2017). Consistent with this, differential abundance analyses of microbial communities from SSB and FWB showed that OTUs assigned to the Fusobacteria, a bacterial phylum known to comprise many pathogenic strains (Bennett & Eley, 1993), were strongly overrepresented in the high density colony. Furthermore, the majority of these OTUs revealed top BLAST hits to species that are known to be associated with a variety of infections as well as cancer in humans (Table S7). However, it is important to stress that, with only two colonies investigated, we are unable to definitively conclude that social stress modulates microbial community structure in fur seals. To investigate this further, the number and diversity of breeding colonies could be increased in order to test for associations between microbial diversity and social density at the population level. A complimentary approach would be to collect blood samples in parallel to skin swabs, which would then allow testing for associations between stress hormone levels and microbial diversity at the individual level.

Differential abundance analysis of the two breeding colonies was furthermore suggestive of an influence of the physical environment on microbial community composition. In particular, a number of bacterial phyla were disproportionately abundant on FWB including several groups that are typically associated with soil and brackish marine or freshwater (e.g. Gemmatimonadetes, Cyanobacteria and Verrucomicrobia; Bergmann et al., 2011; Büdel, 2011; DeBruyn, Nixon, Fawaz, Johnson, & Radosevich, 2011). This pattern makes sense given that a small stream is present at FWB, and is also consistent with the expectation that environmentally associated bacteria should be more prominent at low social density, as when fewer animals are present and body contact between individuals is less common, the influence of environmental bacteria relative to host-associated commensal bacteria should be greater.

We also considered the possibility that differences in overall alpha diversity between the two colonies could simply be a reflection of the increased relative abundance of environmentally associated bacteria at FWB. To test this hypothesis, we focused on the four most abundant phyla – Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria – which do not differ appreciably in their overall relative abundance between the two colonies (97% at SSB versus 90% at FWB). This analysis recovered the same pattern of significantly elevated diversity in the low-density colony, allowing us to infer that differences in bacterial diversity between the two colonies are systemic rather than being strongly influenced by the exclusion of the most differentially abundant environmentally associated taxa. Nevertheless, we cannot yet exclude the possibility that factors other than social density are responsible for these broad sweeping differences.

The potential role of skin microbes in chemical communication

The bacterial fermentation hypothesis of chemical communication predicts that microbial community structure should covary with individual-specific odours (Leclaire et al., 2017; Theis et al., 2013). Comparing our results with those of Stoffel et al. (2015), we find that animals from the two colonies show parallel differences in their chemical fingerprints and bacterial communities, while regardless of colony, mothers and their pups strongly resemble one another

both chemically and microbially. Our results thus hint at the possibility that microbes could mediate mother-offspring recognition in fur seals. Specifically, the microbial and chemical similarity of mothers and their offspring is consistent with phenotype matching, a mechanism by which the own phenotype or one that is learned early during development is a template used for the recognition of relatives (Blaustein, 1983). However, to provide conclusive evidence of a link between the skin microbiota, chemical profiles and mother-offspring recognition, it will be necessary to sample microbes and chemical substances from the same individuals (Ezenwa & Williams, 2014) as well as to demonstrate that fur seal mothers show a preference for the chemical and bacterial profiles of their own offspring relative to those of non-filial pups (Pitcher et al., 2015).

A further complication is that allosuckling is common in fur seals (Lunn, 1992) and can lead to errors in the assignment of mother-offspring pairs in the field (Hoffman & Amos, 2005). Our genetic data identified five incorrectly assigned mother-offspring pairs at SSB, in line with a previous study at the same colony showing that around 10% of presumed mother-offspring pairs are in fact non-filial (Hoffman & Amos, 2005). These unrelated pairs were nevertheless very similar to one another in their bacterial communities. This suggests that the microbial resemblance of mothers and offspring is not necessarily a reflection of genetic relatedness *per se*, but probably results from the transfer of bacteria between females and pups through close physical contact during suckling and when resting next to each other. By implication, our results suggest that if microbes indeed mediate communication between mothers and offspring, recognition errors could easily arise due to spurious patterns of microbial similarity between non-relatives. This insight may help to shed light on the long debated question of why pinnipeds, as well as mammals in general, often suckle alien offspring (Insley, Phillips, & Charrier, 2003; Packer, Lewis, & Pusey, 1992; Roulin, 2002).

The relationship between host heterozygosity and microbial diversity

Finally, a growing number of studies in humans and laboratory animals have demonstrated that host genetic control and, to a larger extent, environmental factors both play a role in shaping the diversity and composition of the host-associated microbiota (Bonder et al., 2016; Davenport, 2016; Kolde et al., 2018; Lassalle et al., 2018; Rothschild et al., 2018; Shaw et al., 2017). In wild populations, many recent studies have established links between environmental and microbial variation (e.g. Bestion et al., 2017; Kohl & Yahn, 2016; Tung et al., 2015). However, much less is known about the relationship between the microbiota and host genotype. Furthermore, although previous studies of model organisms have largely focused on additive genetic effects, it is also known that heterozygosity, a measure of host genetic quality, correlates with fitness variation across literally hundreds of vertebrate species (Chapman et al., 2009; Coltman et al., 1999; Hansson & Westerberg, 2002; Szulkin et al., 2010) including humans (Lyons et al., 2009). Consequently, we hypothesised that high quality, heterozygous fur seal individuals should be better able to exert control over their microbiota. In support of this, we found a significant negative association between sMLH and microbial alpha diversity after controlling for both breeding colony and age class.

Our findings are in agreement with a recent study of sticklebacks showing that individuals with greater heterozygosity at the MHC have less diverse gut microbiota (Bolnick et al., 2014). Such a pattern could be readily explained by MHC heterozygotes being able to recognise a greater diversity of bacterial antigens, although many other immune loci as well as genes with metabolic, structural and signalling roles are also known to be involved in host control of the gut microbiota in mice and humans (Benson et al., 2010; Bonder et al., 2016; Davenport, 2016; Kolde et al., 2018; Marietta et al., 2015; Spor et al., 2011). Furthermore, genetic association studies in humans, mice and fruit flies have identified multiple genome-wide distributed QTL associated with microbial community structure (Belheouane, Gupta, Kunzel, Ibrahim, & Baines; Blekhman et al., 2015; Chaston, Dobson, Newell, & Douglas, 2016; Si, Lee, Park, Sung, & Ko,

2015), leading to the suggestion that variation in host-associated bacterial assemblages may be a complex and polygenic trait (Benson et al., 2010).

Accordingly, we found no evidence for the association between host heterozygosity and alpha diversity being driven by local effects, as might be the case if one or a few of our microsatellite loci were in linkage disequilibrium with genes of the MHC. Furthermore, although g_2 was not significantly different from zero, inbreeding will often be more easily detected through its effect on phenotype than through correlations in heterozygosity at a few marker loci (Szulkin et al., 2010). In support of this argument, a recent study of animals from SSB reported a non-significant g_2 value based on 27 microsatellites that strengthened to become highly significant with around 10,000 single nucleotide polymorphisms (Humble et al., 2018), as was also shown for harbour seals (Hoffman et al., 2014). Consequently, definitive evidence for inbreeding in this species together with a lack of any obvious local effects is suggestive of an influence of inbreeding on host-associated microbial assemblages.

Reconciling patterns at the population and individual level

Finally, it is worth recognizing that our findings point towards contrasting interpretations at the population and individual level. For example, in our comparison of the two colonies, microbial diversity was found to be lower on SSB, leading us to conclude that reduced bacterial diversity may be associated with elevated social stress at the level of the population. However, at the individual level, it appears to be the most heterozygous animals that carry the least diverse bacterial communities. We believe this apparent contradiction may simply be a reflection of different underlying physiological processes. For example, stress-related density effects could potentially be mediated by circulating hormones, while host inbreeding effects are likely to involve the immune system. To disentangle these possibilities, it will be necessary to collect detailed data on host endocrine and immune activity.

Conclusions

Our study combined 16S metabarcoding with genetic analysis of two contrasting seal colonies to uncover marked differences in host-associated microbial community structure at the population-level as well as clear similarities between mothers and their pups at the individual level. We also uncovered a link between host genetic quality and microbial community composition that could have wide-reaching implications for diverse fields. For example, it is widely accepted that low genetic diversity and inbreeding contribute towards the decline and eventual extinction of small, threatened populations (Gilpin & Soulé, 1986; Saccheri et al., 1998). However, our appreciation of these effects has been limited to *direct* effects on fitness components, whereas the current study suggests that low genetic diversity could also potentially have *indirect* fitness effects via changes to the microbiota. Further studies will be required to establish causative relationships between inbreeding, microbial community structure and function, and fitness variation, but our results suggest that microbiome studies have the potential to contribute towards a more nuanced and far-reaching understanding of how genetic diversity affects population health and persistence.

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Data Accessibility Statement

Raw read data is available in the European Nucleotide Archive (ENA) under study accession PRJEB28012 (sample accessions ERS2630382–ERS2630477). The final set of 788 16S rRNA OTU sequences is available on NCBI GenBank under accession numbers MH728000–MH728787. Additional Datasets S1 to S18 are available from the Dryad Digital Repository under the link https://doi.org/10.5061/dryad.cj05t65.

Author Contributions

JIH designed research together with JBW. SG, AJP, JF, JIH and JS performed research. JIH, BAC and JBW contributed new reagents or analytic tools. SG and JIH wrote the manuscript with all authors providing edits and approving the final version.

Table 1. The Antarctic fur seal skin core microbiota, comprising operational taxonomic units (OTUs) that were found in all 96 sampled individuals.

OTU	Phylum	Family	Genus	Abundance
Otu1	Proteobacteria	Moraxellaceae	*Psychrobacter*	17.28
Otu3	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	5.50
Otu22	Proteobacteria	Moraxellaceae	*Psychrobacter*	3.79
Otu4	Firmicutes	Carnobacteriaceae	*Jeotgalibaca*	2.89
Otu2253	Proteobacteria	Moraxellaceae	*Psychrobacter*	2.83
Otu6	Actinobacteria	Intrasporangiaceae	unassigned	2.47
Otu13	Proteobacteria	Moraxellaceae	*Psychrobacter*	2.12
Otu29	Firmicutes	Streptococcaceae	*Streptococcus*	1.60
Otu16	Actinobacteria	Propionibacteriaceae	unassigned	1.29
Otu15	Firmicutes	Clostridiales Incertae Sedis XI	*Tissierella*	0.99
Otu31	Actinobacteria	Micrococcaceae	*Arthrobacter*	0.88
Otu11	Actinobacteria	Micrococcaceae	*Arthrobacter*	0.84
Otu14	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.83
Otu5	Firmicutes	Peptostreptococcaceae	*Clostridium XI*	0.80
Otu26	Deinococcus-Thermus	Deinococcaceae	*Deinococcus*	0.67
Otu18	Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	0.60
Otu19	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.59
Otu78	Firmicutes	Carnobacteriaceae	*Atopostipes*	0.59
Otu17	Bacteroidetes	Flavobacteriaceae	unassigned	0.59
Otu36	Actinobacteria	Microbacteriaceae	*Agrococcus*	0.59
Otu401	Proteobacteria	Moraxellaceae	*Psychrobacter*	0.57
Otu1771	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.50
Otu25	Firmicutes	Carnobacteriaceae	*Carnobacterium*	0.46
Otu32	Firmicutes	Planococcaceae	*Sporosarcina*	0.44
Otu43	Actinobacteria	Acidimicrobiaceae	*Ilumatobacter*	0.37
Otu82	Bacteroidetes	Flavobacteriaceae	unassigned	0.34
Otu72	Proteobacteria	Rhodobacteraceae	unassigned	0.21
Otu145	Actinobacteria	Microbacteriaceae	*Leifsonia*	0.20
Otu488	Actinobacteria	Nocardioidaceae	*Nocardioides*	0.11

Figure legends

Figure 1. Map showing the two breeding colonies from which Antarctic fur seal mother-pup pairs were sampled: Freshwater beach (FWB, blue) and special study beach (SSB, red).

Figure 2. The relative abundance of common bacterial phyla across 96 Antarctic fur seal individuals from the two breeding colonies (FWB: freshwater beach, SSB: special study beach) based on non-normalised OTU counts. For each sample, only phyla with an abundance of at least 1% are shown, so the stacked bars do not always sum to one.

Figure 3. Comparison of alpha diversity estimates (given as effective number of bacterial species) from the two breeding colonies (FWB: freshwater beach, SSB: special study beach). Data are shown separately for adults (mothers) and pups.

Figure 4. Differences in Antarctic fur seal skin microbial community composition visualised by PCoA of weighted UniFrac distances based on CSS normalised OTU counts (see Materials and methods for details). Individuals are labeled according to (A**)** the breeding colony from which they were sampled (FWB: freshwater beach, SSB: special study beach); and (B**)** mother-offspring pair, with each pair being given a unique symbol-colour combination and connected by thin solid line. Asterisks represent mother-pup pairs that are unrelated according to the parentage analysis.

Figure 5. Results of the differential abundance analysis. Circles represent OTUs that are significantly differentially abundant between the two breeding colonies. OTU phylum membership is represented by the different colours. OTUs with a log2-fold-change larger than zero are significantly more abundant at SSB and OTUs with a log2-fold-change smaller than zero are significantly more abundant at FWB. Only OTUs with reliable phylum classification are shown.

Figure 6. Relationship between microbial alpha diversity (square root transformed effective number of species) and individual heterozygosity (sMLH, centered around the mean). Plotted are the raw data together with the regression lines from a linear mixed model in which the effect of heterozygosity on alpha diversity was tested while controlling for both colony and age, including interactions (not significant) between sMLH and breeding colony, and sMLH and age class.