Habitat heterogeneity of the human skin microbiome: A comparison of the dorsal & ventral forearms

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Introduction

The human skin is the largest organ of the human body and provides a microbiome ecosystem for bacteria. Human skin is composed of 1.8 m2 diverse habitats made up of creases, folds, and specialized niches that support various microorganisms (Grice and Segre 2011). Its variety of regions offers a wide array of habitats for microbial bacteria to reside. These various habitats make up environments with different chemical and physical properties. The chemical and physical make-up of the human body also dictates the type of bacteria residing in these habitats. Physiochemical skin features select for different sets of microbial communities whose niches are specialized to these habitats (Grice and Segre 2011). Biogeography of the human body also determines the composition of bacterial communities (Costello et al. 2009). Human skin is divided into biogeographical habitats via the effects of temperature, moisture, and density of hair follicles.

As the human skin provides a diverse combination of regions, the habitat complexity of these "geographic" areas plays a significant role in the bacterial composition. Since the skin varies topographically amongst regions, differing habitats are recognized to support distinct assemblages of microorganisms (Grice and Segre 2011). The topography of human skin is based on skin thickness and density of hair follicles and glands (Grice and Segre 2011). As skin thickness and the density of hair follicles and glands vary throughout the human body, there is a distinction between habitat heterogeneity. Hair density shapes habitat heterogeneity, as it may provide bacteria with more coverage from elements like the sun or temperature variation (Busse et al. 2018). The combination of hair follicles and sebaceous glands affects habitat structure as the gland sits at the base of the follicle and secretes oils that alter moisture and pH levels (Grice and Segre 2011). The density of these two structures ultimately influences the differentiation between habitats as regions with a high density of glands are moister and harbor more bacteria, whereas regions with more hair follicles shelter a wider variety of species (Grice and Segre 2011). These characteristics are important to consider when studying the biogeography of the skin microbiome because they differentiate between areas like the face and the arm. Ultimately, the variation in habitat creates niche-specific bacteria with physiological differences that affect community diversity, composition, and biomass (Oh et al. 2014). We must also consider habitat complexity and its effect on niche specification, and therefore species richness, to accurately map the habitat heterogeneity of the human body.

Comparable to our planet's diversity rules, the human body also has diversity rules that make up our skin microbiome which we can use to predict areas of diversity hotspots. Recent studies have discovered a link between habitat complexity and species richness, and we can use these findings as grounds to formulate a hypothesis on the human skin microbiome. Regions of high topographic complexity like the tropics and the benthos of marine environments contain high species diversity because they harbor challenging abiotic factors that lead to speciation. Speciation is driven by the partitioning of niches via factors like temperature, precipitation, and variability. Topographic complexity drives the increase in niche space, allowing more species to coexist and therefore more richness within the habitat (Allouche et al. 2012). A recent study on the benthos of marine environments provides evidence for this trend since they discovered that coral reefs harbored high species diversity and primary productivity because these areas were more topographically complex (Zawada et al. 2010). Taking these biodiversity rules into account plays a significant role in the composition of human microbial communities as regions of topographic complexity contain biodiversity hotspots and shape species traits, biotic interactions, and range distributions (Badgley et al. 2017). Therefore, the connection between habitat complexity and species richness will assist in the prediction of microbial diversity.

To study the diversity and richness of microbial communities on the human skin, we will compare topographically varying environments throughout the body. A recent study provides evidence for this comparison as they displayed that the human skin offers an opportunity to study the taxonomic and functional compositions of our microbial communities (Oh et al. 2014). As this composition differs across regions, looking at the density of hair follicles and sebaceous glands creates topographically varying regions as dissimilar as rainforests and deserts. (Oh et al. 2014). To display this compositional relationship between habitats, we will study the dorsal and ventral forearms as they offer differing temporal and topographic environments (Grice et al. 2009). The dorsal and ventral forearms are ideal for the study of varied bacterial composition between regions as they offer different spectrums of habitat heterogeneity. The dorsal forearm offers more heterogeneity as there tends to be a higher density of hair follicles and sebaceous glands (NYU Medical Center and School of Medicine 2007). The dorsal forearm also experiences greater exposure to sun and temporal variability. The ventral forearm harbors less topographic complexity and hair density along with decreased environmental exposure (NYU Medical Center and School of Medicine 2007). As these regions vary drastically in habitat heterogeneity, they are the ideal subject for the purposes of this study.

We aim to characterize the relationship of habitat complexity and the level of species richness on the human body. We hypothesize that topographically complex environments produce greater species richness. Using human forearms, we predict that more hairy forearms are more topographically complex environments than less hairy forearms. We also predict that the hairier environments produce more bacteria growth (richness) than less hairy environments.

Methods

The study included 40 total samples of bacteria cultures from human forearms. We collected two samples from 20 individuals, one from the dorsal forearm and one from the ventral forearm. The dorsal forearm was swabbed in a 2.5 cm2 site, 2 in. from the elbow joint. The ventral forearm was swabbed in a 2.5 cm2 site, 2 in. from the elbow crease. We also collected an equal number of samples from men and women, 10 from men and 10 from women. For data collection, we used random sampling where we did not characterize the heterogeneity of individual samples until after data collection. The samples were plated on agar and placed in the incubator within 1-2 hours after collection. All samples were collected and inoculated according to the *Sampling/Inoculation Procedure* within the Human Biogeography Lab document. We assigned each sample with a number between 1-30 and either letters "A" or "B" (i.e., 1A & 1B). The number corresponds to the individual in which we took the sample, while the letter corresponds to whether the sample was taken from the dorsal or the ventral forearm. The letter "A" corresponds to a sample collected from the dorsal forearm, and the letter "B" corresponds to a sample collected from the ventral forearm. The assigned number and letter will also correspond to the photograph taken for categorization of the hair density. Hair density was determined by estimating hair length in centimeters and the percentage of hair coverage within the 2.5 cm^2 sample site. We achieved these measurements by placing a 2.5 cm by 2.5 cm grid on the sample sites and photographing the area. Hair length and density were both estimated after collection via photographs displaying the 2.5 cm^2 sample site. Each sample was categorized on a continuous scale, but we ultimately determined that a more "hairy" and topographically complex environment contained a hair length of 1 cm or greater and a hair density greater than 50%. A less "hairy" and topographically simple environment harbored a hair length of 1 cm or less and a hair density less than 50%. We also determined different species of bacteria by their color, size, edge shape, and shininess. We determined species richness from the total number of morphologically distinct bacteria species on the plate. The total abundance of bacteria grown was determined by the total number of colonies present on the plate. We ran two linear regression tests to better understand our hypothesis and the relationship between species richness and habitat complexity on the human body. A linear regression compared hair density to the total abundance of bacteria. To correlate the dorsal and ventral forearms, we ran a linear regression for both data sets. In both regressions, we performed a log transformation of total bacterial abundance to standardize the data from outliers. Comparing the amount of bacteria growth per hair density on the dorsal and ventral forearms provided better grounds to test our hypothesis and display the relationship between species richness and habitat complexity of the human body. The male and female samples were also plotted separately to look for trends in hair density and bacterial abundance between the two sexes. An ANO-

VA test was performed within a regression analysis to obtain the *p*-value (*Significance F*), degrees of freedom (*df*), and the variation between samples (*F*). This test helped us determine the significance of our results.

Results

A total of 10 different bacterial morphospecies were discovered. This morphospecies set included *large and small yellow, small and shiny pink, large and small white, large brown, rough-edged white with yellow center, smooth-edged with a brown center, rough-edged white,* and *rough-edged brown*. The *large yellow* and l*arge white* bacterial species were most abundant as they occurred in 80% of our plates containing bacterial growth. The rarest species was the *rough-edged white with a yellow center* as it only grew one colony on a single plate. The plates with the highest abundance of bacterial growth were sampled from males. Our results contained three plates with overgrowth, all of which occurred on male samples (9A, 43 colonies, 5 morphospecies), (20A, 100 colonies, 6 morphospecies), (20B, 27 colonies, 4 morphospecies). Linear regression of the dorsal forearm revealed that the relationship between hair density and *log* abundance was insignificant ($F_{1,18} = 0.684$, $p = 0.419$). The dorsal forearm data points also did not significantly fit the linear regression and varied from the linear trendline ($R^2 = 0.366$). Linear regression of the ventral forearm revealed that the relationship between hair density and *log* abundance was insignificant ($F_{1,18} = 0.214$, $p = 0.649$). The ventral forearm data points also did not significantly fit the linear regression and varied from the linear trendline ($R^2 = 0.117$). In the ventral samples, there were seven plates with one or more colonies grown while the dorsal samples only had four. Although both datasets do not show a significant ($p = 0.419$, $p = 0.649$) relationship between bacterial abundance and hair density, the ventral forearm did produce more bacterial growth overall.

Figure 1. Log_{10} of total bacterial abundance as a function of hair density on the dorsal forearm.

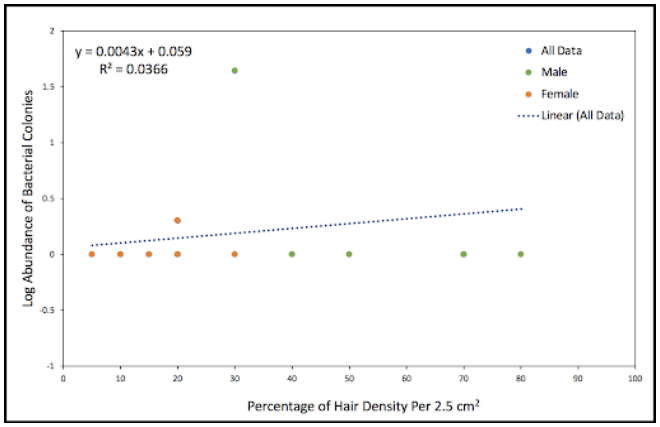

Figure 1 displays the total Log_{10} abundance of bacterial colonies grown amongst varying hair densities on the dorsal forearm. The percentage of area covered by hair per the 2.5 cm^2 sample site is displayed on the x-axis. The Log bacterial abundance grown in each plate is displayed on the y-axis. Male samples tended to have greater hair density and slightly more bacterial growth. Female samples tended to have less hair density and less bacterial growth. The model does not explain much of the variation ($R^2 < 1$) and bacterial abundance as a function of hair density is insignificant ($p > 0.05$).

Figure 2. Log_{10} of total bacterial abundance as a function of hair density

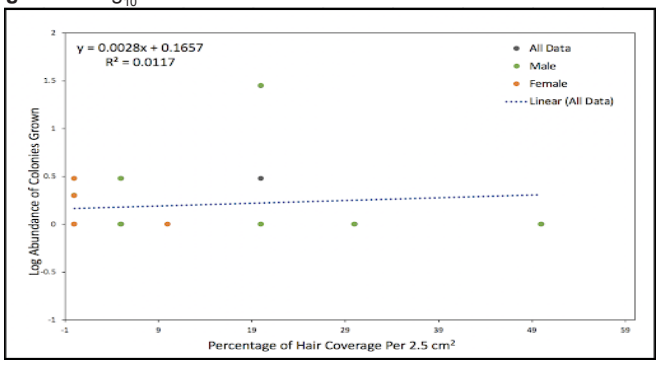

Figure 2 displays the total Log_{10} abundance of bacterial colonies grown amongst varying hair densities on the ventral forearm. The percentage of area covered by hair per the 2.5 cm^2 sample site is displayed on the x-axis. The Log bacterial abundance grown in each plate is displayed on the y-axis. Male samples tended to have greater hair density and more bacterial growth. Female samples tended to have less hair density and less bacterial growth. The model does not explain much of the variation ($R^2 < 1$) and bacterial abundance as a function of hair density is insignificant ($p > 0.05$).

Discussion

We studied the relationship between bacterial abundance and habitat hair density and found no significant correlation between the two variables. Our results from sampling the dorsal and ventral forearms do not support our hypothesis that there will be increased species richness within habitats of high complexity as there was no correlation between the density of hair coverage in 2.5 cm^2 and bacteria richness. Fitting the total bacterial abundance to a Log_{10} transformation did provide different results and did not further support our hypothesis. Our insignificant results may be due to differences in patterns o Although our results did not display a correlation between bacterial abundance and hair density, we did observe patterns between samples taken from the dorsal versus ventral forearm. Analyzing Figure 1 and Figure 2, we see that samples taken from the ventral forearm yielded more plates with bacterial growth than the dorsal region. Since the dorsal region harbors more complexity and habitat heterogeneity, this trend is contradictory as these characteristics are also linked to species richness (Pausas et al. 2013). An explanation for increased growth in the ventral region may be due to immigration from the dorsal forearm. The dorsal forearm is exposed to more sun and therefore more climatic variability, so it may force bacteria to look for a darker and moister region to accumulate. In biogeography, evidence for immigration is provided by the finding that climatic warming leads to range shift of species and pushes them to cooler lowlands (Badgley et al. 2017). This biogeographic trend offers an intriguing insight as our data displayed a similar model on the human body. Looking at the trendline of both Figure 1 and Figure 2, we observe more plates with bacterial growth as hair density increases from moderate to high. Although our data revealed these variables as insignificantly related, the amount of coverage from hair may explain this trend. A recent paper on bromeliad microfauna revealed that canopy cover significantly influenced richness within habitats. They discovered that increased canopy cover led to decreased daily fluctuations in habitat temperature, which allowed for an increased microfauna richness (Busse et al. 2018). Our results align with their findings as we found an increase in bacterial abundance with an increase in hair density. The survival of bacteria is protected from elements like sun and heat because greater hair density provided more coverage.

The majority of our samples taken from both the dorsal and ventral regions did not develop any bacterial growth. The widespread lack of growth may contribute to the insignificance of our data when comparing species richness and habitat complexity. Of the forty samples, only 25% produced one bacterial colony or more. The large lack of growth may be due to the selected habitat. When comparing areas of the body, occluded areas like the groin, toe web, and ear canal tend to have more bacterial growth because they are higher in temperature and humidity. When contrasted with areas like the forearm and leg that are drier and experience more temperature variations, they are found to have significantly less growth than moist regions (Grice and Segre 2011). The discrepancy between these two habitats explains the lack of growth as our samples were taken from a drier, more variable habitat. We also found that we had a fraction of plates with substantial overgrowth as compared to the rest of the samples. Plates that exhibited growth contained 5-10 times more colonies than the other plates, and we discovered that all overgrowth samples came from males. This discrepancy may be accounted for by the biological differences in males and females. As males and females produce different levels of sweat, sebum, and hormones, overgrowth may be because men tend to sweat more and therefore harbor more bacteria (Grice and Segre 2011). Men also tend to have greater hair density than females, which could also contribute to increased levels of sweat and sebum as greater hair density equals more follicles and sebaceous glands (Grice and Segre 2011). Looking at Figure 1 and Figure 2, our data agree with this suggestion because both the dorsal and ventral forearms have greater bacterial abundance with increased hair density in males rather than females. Our results also revealed that our samples produced a wide variety of bacterial morphospecies. This trend is supported by the idea that

habitats containing a greater diversity of microorganisms are typically less stable in member and structure over time. One of these temporally unstable environments includes the volar forearm (Grice and Segre 2011). As the forearm is exposed to sun, heat, and variability, it is more likely to harbor a wider variety of morphospecies because it interacts with more elements.

As a whole, our data reveals that habitat complexity does not play a significant role in skin microbial diversity, and the habitats we chose did not exhibit the level of bacterial abundance and diversity as we assumed. Our non-significant results could also be accounted for by the methods used. Recent evidence has revealed that the swab method we used does not yield an accurate representation of bacterial abundance and diversity (Favero et al. 1968). Some other issues with our methods may include the sample site area, time from collection to plating, and habitat selection. As we discussed above, our choice of the forearm habitat yielded limited growth because it is a drier and more exposed environment than occluded areas as they harbor better conditions for bacterial growth (Grice and Segre 2011). The chemical makeup of our habitats may have also contributed to limited growth because some studies revealed that the microbes residing in sebum and hair follicles are anaerobic. Bacteria of this type wouldn't be able to grow on our plates because they were in atmospheric oxygen conditions (Kong and Segre 2012). In the future, it may be beneficial for our study to compare habitats with more distinct contrast in heterogeneity, for example the armpit and the forearm. The study of skin microbe community composition and assembly is crucial to the identification of patterns in habitat heterogeneity, topographic diversity gradients, and niche specialization. f species richness in wet and dry habitats. Studies have shown that wetter habitats correlate with species richness and harbor significantly more species than drier habitats (Lengyel et al. 2016). This trend may explain our insignificant amount of bacterial growth on the forearm because it's categorized as a dry habitat within the human body.