Looking at the human body's microclimate in a biogeographic context

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Introduction

Species diversity varies across the globe and different environmental conditions. Despite the differences in the number of species between locations, there are measurable patterns relating to species diversity. For example, moving from the poles to the equator, there is an increasing gradient of the number of species present (MacArthur 1965). Typically, the tropics are believed to contain the greatest species diversity because they are composed of many habitats in a relatively uniform climate. The stability of the tropics, along with high habitat complexity, high productivity, and greater competition, are thought to be the driving forces behind the increase in diversity (Pianka 1966). On top of this, it is hypothesized that more uniform environments will have more effective barriers to dispersal than environments with greater fluctuation. This is because organisms that exist in fluctuating environments are more likely to adapt to a greater range of conditions. They are also less likely to encounter unbearable conditions than their counterparts from a stable environment with a smaller range of conditions present (Jazen 1967). As such, organisms from tropical environments are more restricted in their ability to migrate than organisms from temperate climates, allowing diversity to build over time. Establishment of a species in a new location is a rare and difficult phenomenon that gets increasingly unlikely as the distance between locations grows. Dispersal, especially over long distances, is characterized by difficulties involving isolation, ecological opportunities, and climatic differences. Typically, species that are good dispersalists are less competitive and weedier than their counterparts (Carlquist 1966). These species have adapted for movement rather than being able to outcompete other individuals and species. Dispersalist species would be unlikely to successfully establish a new population in a location already inhabited by other species. Rather, they would need to find an empty or sparsely populated area to colonize, which are few and far between. Meanwhile, species that are adapted for survival rather than dispersal are unlikely to survive the journey to a new location and will not migrate or expand from their original range without some form of intervention.

On average, the human body is home to trillions of individual microbes and thousands of species (Hulcr et al. 2012). The human body can be considered a planet with each individual harboring unique microbial communities that live in a range of physiologically and topographically distinct niches. Much like how the Earth is broken down into different biomes based on environmental conditions, human skin is broken down in different regions based on physiological characteristics. Skin is generally characterized as one of three types: sebaceous, wet, or dry (Byrd et al. 2018). Therefore, the human body can be thought of as at least three different biomes. These biomes can then be compared to preexisting environments on Earth. Moist, hairy underarms can be considered analogous to tropical rainforests, while smooth, dry forearms are comparable to desert areas (Grice et al. 2009). Known patterns about Earth's species diversity can then be applied to the human body to hypothesize how microbe richness and abundance may vary between each body part. To explore and test the dynamics of microbes on the surface of the human body, species presence and amounts were recorded on three different parts of the human body: the armpit, the elbow crease, and the back of the knee. We hypothesize that the armpit is a warmer and more constant environment compared to the knee and the crease of the elbow. Since the armpit is a more constant environment, we predict that there will be greater species diversity. This hypothesis is based on the tropics being a highly diverse ecosystem with which the armpit shares many qualities, such as high humidity, complex environment, and relatively constant conditions. Our second hypothesis is that isolation between body parts would decrease similarity in microbe species. Therefore, we expect the elbow to have more similar microbes to the armpit because they are closer in proximity to each other than the back of the knee is to either of them.

Methods

For this study, we collected swab data from 15 participants, sampling their armpit, back of the knee, and elbow crease for a total of 45 samples. The participant pool we sampled from were males at Lake Forest College who use antiperspirants and do not shave their armpits. Additionally, all participants were questioned on their shower frequency per week, how recently they showered and applied antiperspirant, and their amount of regular contact with chlorine chemicals. The 15 participants were swabbed following the lab handout except for the duration of the Q-tip swab. Rather than 3 rotation cycles, participants swabbed each location for 15 seconds. For each sample location, participants swabbed an area of 2.5 cm at the center of each site, approximately the area of a quarter. Each sample was recorded with either the letter A for the armpit, E for the elbow crease, or K for the back of the knee. This was followed by numbers 1 through 15, which corresponded with the participant's number. Participants' names were recorded along with their number on a separate document for anonymity. Additionally, the initials KM were added to each label to differentiate the samples from other experiments.

Each participant conducted the swabbing themselves following the instructions outlined above. The amount of time between sample collection, application to the petri dish and storing the samples in the incubator was approximately one hour. We followed the inoculation protocol provided for each sample and stored the plates in the 36°C incubator for 3 days before taking them out and wrapping the edges of the sample plates with parafilm to be stored in the fridge. Each day within the 3-day span, we checked the plates at about 6:00 PM to observe growth in the incubator. After one day of cooling in the fridge to ensure no residual growth, the samples were photographed for analysis and placed back into the fridge. The photographs of each petri dish were examined to measure the number of species and number of microbes present in each. Overall, we differentiated species using morpho-species characteristics of size, shape, texture, and color. All the data were recorded in an Excel spreadsheet and compared in averages using a one-way ANOVA of three body parts for statistical analysis. We ran separate ANOVA tests for each of the following measurements: species abundance, diversity, and richness from the data. Diversity was measured using the Shannon Weiner Diversity Index and the results were analyzed for significance using a one-way ANOVA. We also completed six t-tests to examine the statistical difference between our two most plentiful species in each location.

Results

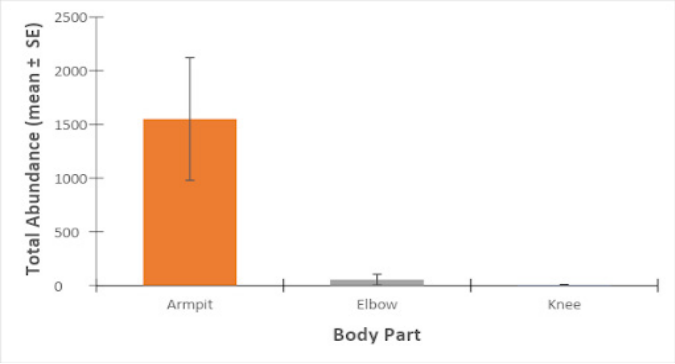

Figure 1. **Total average abundance of species located at each of the three body locations.** The mean was calculated for each body location (armpit, elbow crease, and back of the knee). Error bars measure the standard error of each total average abundance. Statistically significant by ANOVA: $F_{2, 42} = 7.03$, $p^* = 0.002$.

Total average abundance was calculated to determine which of the three locations measured had the most overall microbes. This data was found to be significant (ANOVA: $F_{2, 42} = 7.03$, $p^* = 0.002$) with greater microbe growth located in the armpit compared to the elbow and knee, which is reflected in Figure 1. Total abundance was calculated by taking the average of the counted microbes on each body part. Species richness (ANOVA: F2, 42 = 1.84, p= 0.171) and species diversity (ANOVA: $F_{1, 4} = 0.42$, p= 0.550) were also calculated, but both data were found to be insignificant.

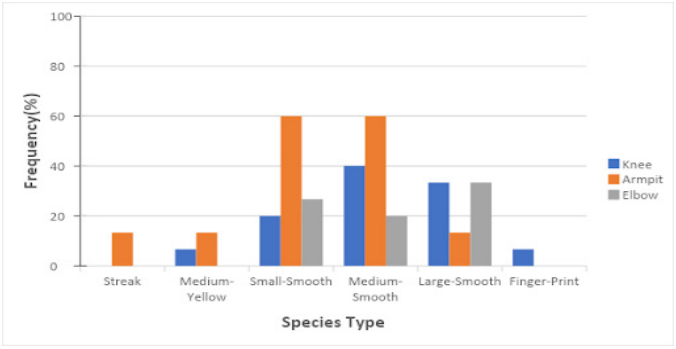

Figure 2. **The frequency of each species recorded from counting the number and type of species from 45 samples.** Descriptions of the distinguished species are along the x-axis based on the researcher's view of distinct morpho-characteristics. The y-axis denotes frequency [%].

Frequency analysis of the six species present in the forty-five samples taken showed that the armpit and the knee had the maximum species types (5 out of 6), while the elbow samples only contained 3 out of 6 (Figure 2). The most common species across all three locations were the small-smooth, medium-smooth, and large-smooth circles. Large-smooth circle distribution was found to be insignificant (ANOVA: F2,42 = 0.05, p= 0.949) while the medium-smooth circle distribution (ANOVA: F2,42 = 3.38, p*=0.044) and the small-smooth circle distributions (ANOVA: F2,42 =6.88, p*= 0.003) were found to be significant. Three t-tests were performed between each of the locations on the body for both the medium-smooth and small-smooth circles. Each of the medium-smooth location comparisons were not found to have significance (Paired t-testAE: tdf = 1.73, p= 0.105, Paired t-testAK: tdf = 1.92, p= 0.076, Paired t-testEK: tdf = 1.92, p= 0.076), indicated by the mean abundances measured in Figure 3. Of the small-smooth circle data, comparisons between the armpit and the elbow (Paired t-test: tdf = 2.54, p*= 0.024), as well as the armpit and the knee (Paired t-test: tdf = 2.68, p*= 0.018), both were found to be significant, while the compared abundances between the elbow and knee were insignificant (Paired t-test: tdf = 0.97, p= 0.346), as shown in Figure 4. The small-smooth circles had greater preferential abundance on the armpit than both the armpit and the knee.

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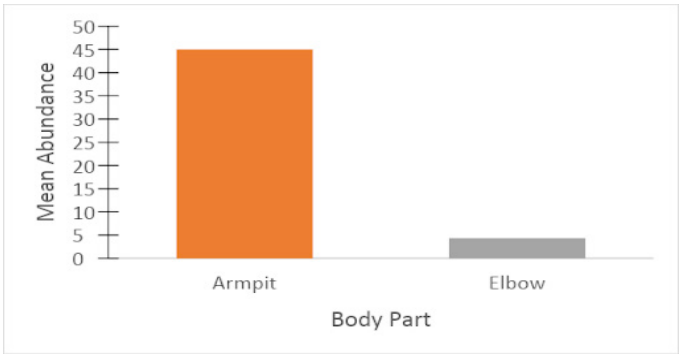

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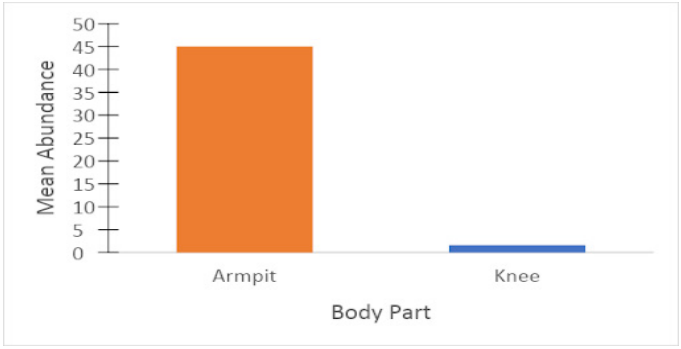

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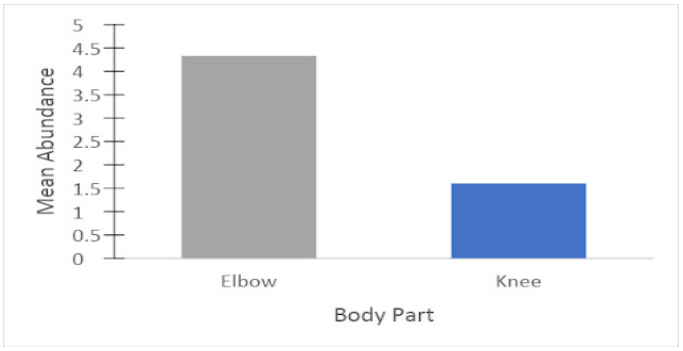
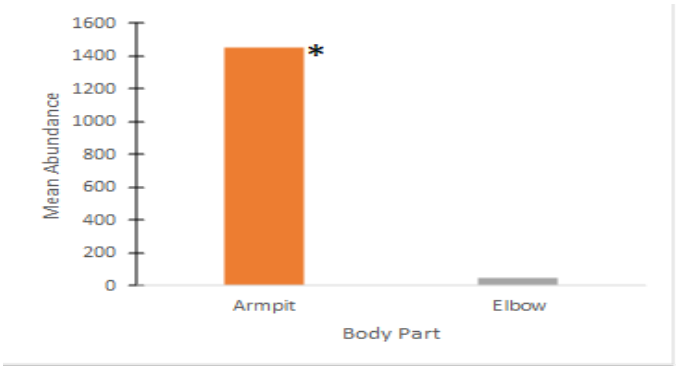


Figure 3. **Mean abundance of medium-smooth circular species.** Comparison of medium smooth species between (A) armpit and elbow, Paired t-test: tdf = 1.73, p= 0.105; (B) armpit and knee, Paired t-test: tdf = 1.92, p= 0.076; (C) elbow and knee, Paired t-test: tdf = 0.63, p= 0.542.

A



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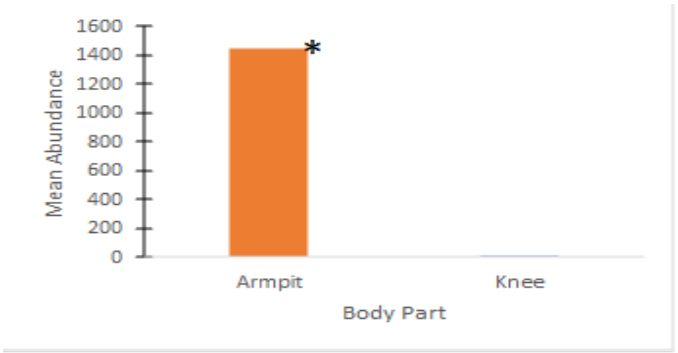

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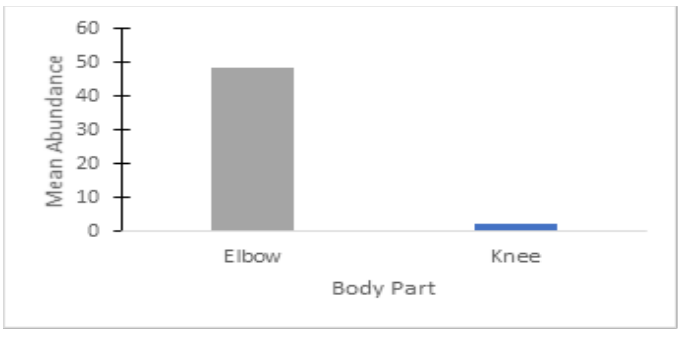

Figure 4. **Mean abundance of small-smooth circular species with significant data indicated by *.** Comparison of differences between (A) armpit and elbow, Paired t-test: tdf = 2.54, p*= 0.024; (B) armpit and knee, Paired t-test: tdf = 2.68, p*= 0.018; (C) elbow and knee, Paired t-test: tdf = 0.97, p= 0.346.

Discussion

While the hypotheses posited that the armpit would have the greatest amount of species diversity, species richness data were not found to be

significant. However, despite the insignificant species richness value, the armpit was found to have the highest frequency of species (Figure 2) and the greatest total abundance (Figure 1) of the three sampled locations. Additionally, the armpit did have the greatest calculated diversity index. However, this also was not found to be statistically significant. Based on these results, we reject the hypothesis that the armpit would have the greatest species diversity due to its warmer and more constant climate. We also cannot accept the hypothesis that isolation between body parts would decrease similarity in species diversity. This is due to a lack of significant results when comparing species richness and abundance between locations. The mean abundance only produced significant results for small microbes between the armpit and elbow and the armpit and knee. All other comparisons between locations and other species produced nonsignificant results. While much of the data proved to be statistically insignificant, an interesting trend involving the frequency of certain species was discovered. It was observed that the medium-smooth and small-smooth circular microbes had a greater presence than other species found in the samples. Hucler et al. (2012) found a similar trend with the bacterial phylotypes present in the human belly button, with the most frequent and abundant phylotypes being present across independent populations. These frequent and abundant phylotypes were termed oligarchs, the same as species found to be both predictably frequent and abundant in tropical rainforests. While oligarchs were present in multiple samples, not one oligarch was present in every sample, much like what we found with our medium-smooth and small-smooth circular microbes. Hucler et al. (2012) determined that these oligarchs allow for some degree of predictability while the rest of the system is made up of randomly determined microbes.

This study was highly restricted due to a variety of limitations as well as sources of error. The chief limitation was the limited sample population swabbed, with only 15 individuals being tested, resulting in a total of 45 samples. More participants and a larger sample size may have produced more microbe variety. Our gut microbial communities stabilize around three years of age, and the strains within the gut likely come from close contact and family members, which are then maintained throughout life (Byrd et al. 2018). The skin may undergo a similar process, and therefore individuals from different regions may have different microbial communities on their skin. Additionally, while shaving practices and the use of antiperspirants were controlled for, the type of antiperspirant and use of other chemical products were not controlled. The differing chemical makeup, addition of other products, or time of product application could have affected the sample's microbe makeup. Finally, relying on self-designed morpho-characteristics to distinguish species type most likely affected the total number of species recorded and therefore skewed the diversity data. Further research into the microbe diversity of human skin remains both an intriguing scientific venture and medically beneficial. Characterizing the microbes that inhabit specific body parts may provide more insight into the balance between human health and disease. Human skin has high variability at different points in time, though there are some common areas of the human body where certain microbe taxa and diversities are consistently located (Ursell et al. 2012). This variability limits what we know overall and could be causing different medical and health issues. Antibiotic exposure, modified hygienic practices, and lifestyle changes have the potential to alter the skin microbiome selectively (Grice et al. 2009). The altered skin microbiome has many results, such as an increased frequency of human skin conditions or removal of a protective barrier unknown to us. Understanding the ecosystem that lives with us every day can also provide insight into the condition that led to the emergence of antibiotic-resistant organisms.