Testing human skin's microbiome to determine species diversity in a biogeographical context of intermediate disturbances: makeup as a disturbance factor

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Introduction

Skin is the biggest human organ and is the most exposed to the external environment (Todar 2004). The exposure makes human skin home to a number of microorganisms including bacteria, fungi, and viruses (Byrd et al. 2018). All those microorganisms form microenvironments all over the human body and are part of the skin microflora. Human skin is an ecosystem consisting of microbial communities distributed in the range of niches defined by external physiology (Grice et al. 2009). Human skin can be categorized into three types of microenvironments: sebaceous, moist, and dry (Byrd et al. 2018). Altogether there are four bacterial phyla identified to reside on human skin: Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes (Byrd et al. 2018). Numerous findings on the human skin microbiome made it possible to look at the human body as a possible analogy for biogeographical patterns found in nature, and the rest of the phenomenon seen in the environment. As the ecosystems differ across the globe due to both biotic and abiotic factors so is human skin predicted to differ from human to human. As the biggest organ, the human skin is daily exposed to the use of synthetic and natural ingredients contained in soaps, gels, moisturizers, and a variety of cosmetic products. They act as external factors that influence a human microbiome to differ depending on products used, continuity of use, and functionality of the product. Therefore, species diversity and richness in microenvironments are both affected to a testable extent since the human skin and the biogeography of the environments are seen to be very similar (Datir et al. 2020).

Biomes in nature differ in defined biotic and abiotic factors; therefore, the flora and fauna of the given biomes respond and recover differently after disturbances. For example, the temperate biome differs from the tropical biome through climatic stability level, seasonality, precipitation fluctuations, and temperature (Dobzhansky 1950). However, tropics are known as the most diverse systems in the world (Harvey et al. 2020). The tropics undergo mild disturbances that keep the equilibrium and nonequilibrium shifting and therefore keep the diversity at a high level (Connell 1978). Since the exposure to the disturbances can be either short or long-term and can range in frequency, it significantly influences the effect (Odum 1963). Connell in 1978 modernized and looked into the idea of equilibrium and nonequilibrium shifts in tropics and coral reefs with the respect to the impact on biodiversity and species richness. He introduced the intermediate disturbance hypothesis proposing the idea of a system experiencing gradual changes in order to keep the environment in the nonequilibrium state where the species diversity itself is the highest. On the contrary, he proposed that the lack of disturbances keeps the system in equilibrium where the diversity is low, while too much and/or constant disturbance can cause exclusion of unadaptable species and further decolonization. Considering the now widely accepted ecological principle of intermediate disturbances, I found it compelling to test it on human skin described as a valid and compatible tool to show biogeographical patterns.

I will question the effect of makeup application frequency on species abundance, species richness, and species diversity of the microbiome of the microenvironment of the human skin, eyelid specifically. It is assumed that abiotic factors will remain as static as possible since the microenvironment is kept constant, but the biotic factors and disturbances will vary due to makeup utilization. I predict that the group of participants that apply makeup at the rarest frequency, compatible with tropical areas with low disturbances, will have low species diversity due to a lack of disturbances that keep the system in equilibrium. The group that often applies makeup is predicted to have the highest species diversity because it is associated with intermediate disturbances in the tropics. The final group, the third group, is compatible with tropical areas that are constantly exposed to disturbances and is predicted to have lower diversity and richness than the intermediately disturbed systems but greater diversity and richness than areas with the lack of disturbances. Further, I predict that if the diversity and richness in group three are higher than expected the microenvironment is in the early disturbing stages. While, if it is lower than expected, the microenvironment is adapted to disturbance and is near equilibrium. Thus, I hypothesize that often makeup application causes intermediate disturbances in the eyelid microenvironment that results in high biodiversity and species richness in the eyelid microbiome.

Methods

The study was done among students that identify as women at Lake Forest College, Illinois in April 2022. For the purpose of this experiment, makeup was characterized as the source of the disturbance in the microenvironment of the human microbiome on the eyelid. The eyelid specifically was chosen because I assumed it to be the most makeup-exposed area among participants. Participants were divided into three groups based on the frequency of makeup application, and while gathering the samples participants were questioned on their frequency of make-up use over time. In advance, we agreed to look for three groups ranging as follows: group one participants wears makeup up to 5 times a year/never wears makeup (G1), group two participants wears makeup 2 to 3 times a week/often wears makeup (G2), and group three participants wears makeup more than 3 times a week/always wears makeup (G3). Group 1 was set to be compatible with tropic environments that are in equilibrium due to rare disturbances. Group 2 accounts for intermediate disturbances and a nonequilibrium microenvironment system. Group 3 associates with environments that are constantly exposed to disturbances. In our study source of the disturbance was considered make-up, and the frequency of make-up use was considered as the continuity of disturbance. Each group contained 13 samples, which resulted in 39 data samples overall. There were no set rules for the participants to either have makeup on or not at the moment of the sample collection. However, all the participants from group 1 and group 2 did not have any makeup at the moment, while all the participants assigned to the third group had eye makeup. Since not all the participants' data was collected on the same day, I marked each sample by assigning the group, number of the sample within the group, data when the sample was collected, and the time when it was collected. This information was also assigned to any further use of the given sample (e.g., PBS tube, and petri dish) for the purposes of unexpected errors so the sample could be reused if needed. All the data samples were collected by sampling only one of the participant's eyelids because I assumed no difference between the microenvironment of the two eyelids on one individual.

The sampling was done using a cotton swab in circular motions with gentle pressure on the eyelid for approximately 10 seconds. When the swab sample was taken, the cotton Q-Tip swab was soaked in PBS solution contained in a 0.5 mL PBS tube. The tip has stirred the solution between 10 to 15 seconds to yield as much of the sample. After each set of data was collected, the samples in the PBS were placed in the fridge for 3 hours from the time marked on the sample tube. Further, I followed laboratory protocol and shake the PBS solution in the tube well before plating 50 uL of the sample onto the surface of the TSA plate. The rest of the solution from the tube was saved and taken back to the fridge. Then, approximately 5 sterile glass beads were rotated in the TSA plate to spread the suspension all over the agar for 15 seconds. When the given set of samples was all plated, each plate was closed and then taped with paper tape to be secure. The plates were placed upside down (the agar side was down) in the 36-celsius degree incubator for 72 hours counting from the time marked on the petri dish. After the set time, plates were taken out and photographed under the magnifier two times, once without the Petri dish cover, and the second time with the information contained on the petri dish cover. That record procedure was for the purposes of the further analysis of the qualitative data through the pictures. Each plate was analyzed based on the bacterial colonies that formed considering the individuals on each plate. The information on the morphology of the data that was gathered was entered into ExcelSheet recording the following information: a) a number of colonies (both overall and then individuals within the species), b) approximate size of each individual, c) color of the colony, and d) any other visual feature or the characteristic that we considered valid for the study and data. In this study, I considered individuals to belong to the same species if they were similar in physical appearance including the same color, similar size, similar shape, and other possible physical features.

Based on that I distinguished between morphological species and used that data to conduct quantitative statistical analysis. Statistical data analysis included ANOVA statistical tests for each group of the study for their species abundance, species richness, and finally species diversity. The species diversity index was done using the Shannon diversity index check.

Clean-up for the experiment consisted of separate beakers for used material including pipette tips, Q-tips, and glass beads. When plates were fully analyzed, and the experimental part of the study was done, plates were disposed of following laboratory procedure.

Results

In calculating the mean abundance, we found that the greatest microbe growth was found in females that always apply makeup, followed by the group that never applies makeup. The least species abundance was found in the intermediate group that often wears makeup (Figure 1). For the analysis, the results were log transformed to reduce the skewness of the original data, but were not determined to be statistically significant (ANOVA: F(2,36)=[0.148], p=0.86).

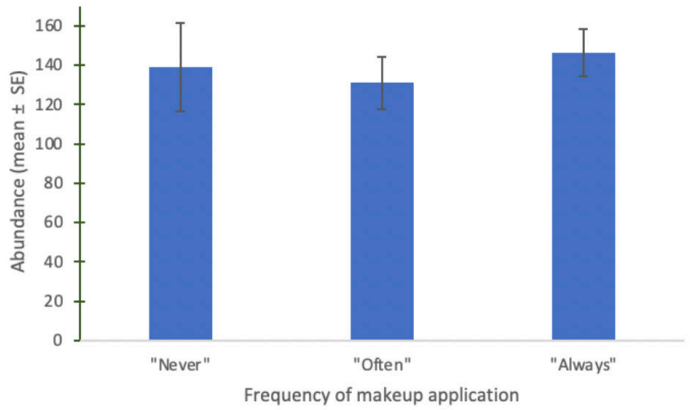

Figure 1. Mean abundance of species. The x-axis displays the frequency of makeup application based on three categories: "always", "often", and "never" wearing makeup determined by the researchers. The y-axis shows species abundance by counting the colonies on each plate and determining the mean.

Moreover, the group that always applies makeup has the smallest species richness ($\bar{x}$ = 1.54) whereas the other two groups have approximately the same mean of 1.69 (ANOVA: F(2,36)=[0.149], p=0.86). Additionally, the species diversity index was determined and it was calculated to be statistically insignificant (ANOVA: F(2,36)=[1.118], *p=0.34).

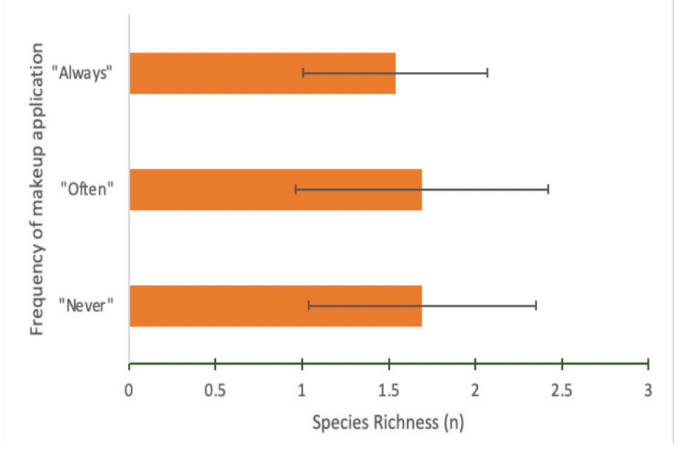

Figure 1. Species richness recorded in 39 samples. Species richness measured by counting the number of different species in each group are displayed on the x-axis and the y-axis shows the three groups tested: "always", "often", and "never" wearing makeup.

There were 8 different microbes identified in the three different groups: large white, small white, small yellow, large yellow, fungi, orange, brown, and pink. The group that never applies makeup had all the species types present, whereas the group that always wears makeup had only four microbes present from all the 13 samples (large white, small white, small yellow, and large yellow) which are also the most abundant species across all the three groups (Figure 3). The group that often applies makeup had all of the microbes except for one (brown) which was unique to the group that always wears makeup.

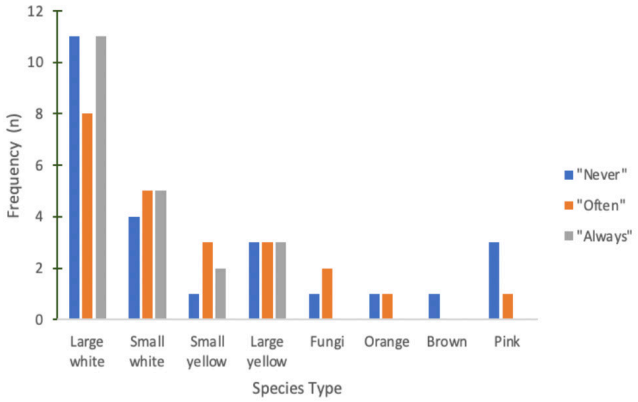

Figure 3. The frequency of each species recorded from the number and type of species. The three different groups tested are along the x-axis and the y-axis displays frequency. The legend shows descriptions of the morphologically different species based on the researcher's observations.

Lastly, the abundance of the most common species was calculated. Figure 4 shows that the large white species have the smallest abundance in the intermediate group that often wears makeup, followed by the group that never wears makeup. The largest abundance is found in the group that always wears makeup with a mean of $\bar{x}$ = 99. (ANOVA: F(2,24)=[1.520], p=0.24). For the small white microbes, the results displayed a decrease in the abundance from the group that never wears makeup to the group that always wears makeup, with the mean changing from $\bar{x}$ = 313.8 to $\bar{x}$ = 140.6 (ANOVA: F(2,13)=[0.870], p=0.44).

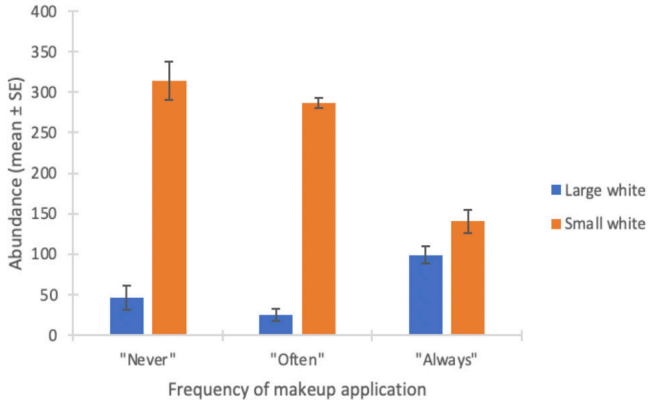

Figure 4. The mean abundance of most frequent species: large and small white. The x-axis displays the frequency of makeup application and the x-axis is the mean abundance.

Discussion

Although the hypothesis proposed that the highest biodiversity of the eyelid microbiota out of three experimental groups is associated with often makeup application, compatible with intermediate disturbances, the results opposed it. The species diversity index was determined by compromising species abundance and species richness data, and it was calculated to be statistically insignificant (p=0.34). Thus, the hypothesis of high diversity being maintained when the frequency of the disturbance is intermediate is rejected. Further, results indicated that species abundance was the lowest in the second group compared to the rest two (Figure 1), while it had the highest species richness together with the third group (Figure 2). However, both results for abundance and species richness were shown to be insignificant too (p=0.86, p=0.34),

which makes them incompetent with the study predictions. Hence, compared to the study of intermediate disturbances in tropics and coral reefs (Connell, 1978), my study differed in the results and found the intermediate disturbances hypothesis not to be applicable to the human microbial microenvironment.

However, the emphasis is that my study was greatly restricted by the number of samples taken and therefore limited the validity of the results. Despite this, certain trends in the study, although found insignificant, are worth the discussion, considerable further improvement, and the potential subject of reinvestigation in the future. Aware of the insignificancy of the results, the study revealed some unexpected outcomes that could be analyzed in contrary to prior predictions in biogeographical context. One of the predictions was that constant disturbances maintain higher diversity than the absence of the disturbances, which was seen to be opposed by the results. The valid explanation is that environments get adapted to disturbances when they are continuous over the time (Connell 1978). Consequently, the system reaches an equilibrium and the diversity is then lower (Connell 1978). The low diversity in the tropics is characteristic for tropical areas with lower temperatures and precipitation but greater seasonality change and larger climatic change over the course of the time (Harvey 2020). In the context of the human eyelid microenvironment, it would assign to drastic change going from undisturbed to by makeup disturbed skin among the participants that use makeup more than 3 times a week/always. Tropical environments with low diversity are usually subject to young habitat with recent colonists, mostly invasive, that would account for external colonies introduced with the makeup utilization (Harvey 2020).

Moreover, the group with the rarest makeup application frequency, compatible with rare disturbances, had higher diversity than expected. Results were possibly affected by unknown and untested external or internal disturbances, e.g., use of cosmetics other than makeup, individual diet, or hygiene (Wallen-Russel 2018). Recent studies that included more products than makeup revealed that the use of synthetic cosmetics on a human face affects the consistency of microbiota of human skin (Datir et al. 2020). Cosmetics can be damaging to human skin and cause numerous skin diseases and allergies which indicates the sickness of the microenvironmental system (Moskovicz et al. 2020). Hence, the healthier the system is, the greater the biodiversity it has (Leftcheck et al. 2015). The health of the environment is also described by the type of species present, meaning the system is healthier and tends to be more diverse when there are less invasive species present (Byrd et al. 2018). In the context of our study, rare or often makeup applications disturbed the microenvironment to the extent where it is not damaged and it can maintain high biodiversity. Thus, the decrease in skin exposure to synthetic ingredients increases microbial diversity and species richness (Wallen-Russel 2018). Wallen-Russel's study (2018) is valid to compare the results since he used a similar methodology to measure alpha diversity where the microenvironment was kept constant while the skin cosmetic exposure was changed respectively.

Further, the morphological dynamics within the groups indicated that group 1 had eight (the highest number) morphologically distinct species out of the three groups, followed by group 2 which had seven of them, and group 3 had only four of them (Figure 3). The logistics behind is that the extreme environments, considering tropical areas with low to no disturbances, enhance the opportunities for speciation which explains why group 1 had all the morphological species present (Harvey et al. 2020). In contrast, moderate environments reduce speciation but permit diversity to accumulate (Harvey et al. 2020). In addition, if it is considered that in each group all the species were equal in competitive ability in distinctively disturbed environments, species that were most resistant to damage filled much of the space and potentially eliminated the rest of the species (Connell 1978). Therefore, four morphological species present in group 3 are all present in two other groups, and one morphological species (brown) is only present when there are no disturbances (Figure 3). Group 1 and group 2 have a higher abundance of large white species than small white species, compared to group 3 which does not have that drastic difference between those two species in abundance (Figure 4). Based on observations, white large bacterial colony species are the dominant species in group 1 and group 2 (Figure 4). One species dominance is associated with the lack of the disturbances rather than any other parameters (Connell 1978).

However, the idea of specific species presence being correlated to the frequency of makeup application would require expanded investigation itself with a more complex methodology. The DNA test to identify the specie would be much more accurate than identification done based on physical features in this study. Because there is a high chance of identifying the distinct bacterial species as the same ones since some look very similar. Also, the use of DNA would help in identification of species taxonomy since more variance is observed to be at lower taxonomic levels (Byrd et al. 2018). Moreover, considering both internal and external factors in the past resulted in more adequate conclusions (Levy et al. 2017). This study also did not consider the intensity of the disturbance that comes in the amount of makeup applied, nor did it consider the time for how long makeup approximately stayed on the eyelids of the participants. Therefore, future studies could expand and improve factors that limited my study, but there are also other interesting topics to be tested in the context of biogeography and the human microbiome. For instance, future studies could focus on eyelids as island like systems and test various hypothesis in that context. Finally, different results might be found on different human body parts including different microorganisms species and colonies shaping the diversity.

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