Research reports from undergraduate students receiving support from the Shackouls Honors College via the Summer Research Fellowship

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Project Title: Reconstruction of host immune status, host immune response (inflammatory phenotype), and heterogeneity in frailty to acquired syphilis.

Description of Project

Biological anthropologists rely on skeletal data to understand how biology and culture have interacted to shape the biology of past human populations, including their experiences of health and disease. This is especially true when historical records are biased, limited, or entirely absent. In this project, investigators examine how experiences of chronic stress, age, nutrition, pre-existing and co-morbid health conditions (e.g., periodontal disease (PD)), and immune status within human hosts (i.e., host characteristics) relate to the trajectory and severity of infection with acquired syphilis (*Treponema pallidum pallidum)* within pre-antibiotic era, late 19th to early 20th century individuals with antemortem, clinical diagnoses of syphilis curated in historical documented skeletal samples. The project will advance knowledge about factors that influence vulnerability to infectious disease within past populations, enabling more accurate estimations of disease burden in the past and therefore more empirical translations between interpretations of health and disease in the past and its patterning within present-day populations. The research findings may also inform clinical guidelines for the screening and diagnosis of modern cases of syphilis to more accurately identify host characteristics that are likely associated with persistent infection into tertiary syphilis.

In this NSF-funded project[1], the research team investigates host characteristics potentially associated with the resolution of syphilis infection, and recovery from syphilis, after the early stage of the disease (i.e., primary, secondary syphilis) and those associated with persistence into late stage (i.e., tertiary) infection, which causes destructive and debilitating symptoms and is sometimes fatal. The investigators will combine skeletal and historical data for over 300 skeletal individuals in the Hamann-Todd Osteological Collection (HTOC) in Cleveland, OH (summer 2022) and the Robert J. Terry Anatomical Skeletal Collection in Washington, DC (summer 2023) to: 1) determine whether chronic stress, nutritional status, age, and co-morbid disease conditions are associated with persistent syphilis; 2) examine whether excessive inflammation in response to other disease causing agents, such as those which cause PD, is associated with late stage syphilis; and 3) develop a multi-stage interpretive model for biological anthropology studies of syphilis that would enable the reconstruction of a human host's immune status during early-stage infection, as well as their immune response during late-stage infection.

Research Performed

Within this, my independent research investigates how experiences of health, disease, and chronic stress over the life course impact an individual's risk of developing late-stage syphilis and various symptoms of syphilis, as evident in the skeleton and historical medical data associated with individuals in the above samples. During the summer, I traveled to the Smithsonian's National Museum of Natural History and recorded evidence of five skeletal and

oral biomarkers of stress and immune competence. Specifically, I recorded evidence of PD, which reflects an impaired adult immune system; linear enamel hypoplasia (LEH), which represents arrested growth in tooth enamel, indicative of episodes of substantial physiological stress during growth and development; dental caries/ cavities, which reflects an impaired immune system; and length of the femur and width of the femoral head, which reflect physiological stress during growth and development and affect stature. I followed established standards (Marklein et al. 2016, Powers [2008] 2012). Together, these five biomarkers make up a 5-biomarker index for estimating stress over the life course within the Skeletal Frailty Index (SFI). The SFI is an index-based established tool within biological anthropology for estimating overall health and differential vulnerability to death and disease (i.e., frailty) in past individuals. Importantly for my independent research, and the larger project, it is also designed to enable direct comparisons to index-based measures of health and frailty in modern populations. For my hypothesis, I predicted that skeletal and/or historical evidence of late-stage syphilis will be associated with these five biomarkers; in other words, progression to late-stage syphilis, and the inadequate host immune response that this progression indicates, will be associated with poor overall health and experiences of chronic stress over the life course and an associated impaired immune response. To accommodate small sample size, associations between individual biomarkers and syphilis stages I am comparing mean indices for this 5-biomarker SFI between individuals with documented and/ or skeletally evident early-stage syphilis *vs.* late-stage syphilis. As tertiary syphilis is more likely to occur in older adults and as some components of the SFI might also be associated, positively or negatively, I am controlling for age using ANCOVA, with age as a covariate. To accommodate small sample size, associations between the individual biomarkers (e.g., LEH) and syphilis stages will also be assessed, again controlling for age, using

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ordinal logistic regression. To accommodate small sample size, associations between individual biomarkers and syphilis stages will also be assessed using ordinal logistic regression. I will use Bonferroni corrections for both tests to counteract the use of multiple comparisons. These analyses and tests follow established methods, as they can identify significant differences in variables (i.e., biomarkers) between groups (i.e., early *vs.* late-stage syphilis), even when there are relatively few differences. Evidence in support of my hypothesis would consist of a higher 4-biomarker-SFI value in those with late-stage syphilis than in those with early-stage, indicating that stress and disease over the life course likely facilitated the development of persistent, late-stage syphilis through an impaired immune response.

This analysis follows established methods (Cheverko and Hubbe 2017), as they can identify significant differences in variables (i.e., biomarkers) between groups (i.e., early *vs.* late-stage syphilis), even when there are relatively few differences. Evidence in support of my hypothesis would consist of a higher 5-biomarker-SFI value in those with late-stage syphilis than in those with early-stage, indicating that stress and disease over the life course likely facilitated the development of persistent, late-stage syphilis through an impaired immune response.

Impact of Experience

This study addresses a key question in biological anthropology, clinical medicine, and public health about variation in syphilis' outcomes: what characteristics can lead to a patient having a more severe case of the disease and progressing towards destructive, debilitating, and sometimes fatal late-stage syphilis? This question persists, largely unanswered, because it is very difficult to investigate syphilis and *T. pallidum* empirically using traditional microbiological methods and data (i.e., culturing *and in vitro* analyses, animal models, genetic tools for genome

modification, human experimentation) (Partieli & Yiasemis, 2021). Rapid increases in syphilis'
global incidence make these findings critical; to date, genetic, animal model and human studies
have not identified host characteristics that may generate differential frailty to syphilis. In
addition to furthering understandings of syphilis, which has become dramatically re-emergent in
global, present-day populations, this study more generally advances analyses of variations in the
disease's progression, a crucial--but neglected--analytical issue in biological anthropology that
complicates reconstructions of past health. Through use of recent historic populations, the SFI
and translational medicine, findings can also be used to refine guidelines for screening and
diagnosis of syphilis cases to more accurately identify patient/ host characteristics likely
associated with persistent infection; mild and/ or atypical early-stage cases are often
misdiagnosed, leading to failed treatment, continued transmission, and late-stage disease. My
independent research will directly contribute to these two aims; findings from my analyses will
contribute to more accurate understandings of stress and disease over the life course contribute to
an individual's vulnerability to persistent, late-stage infection; these findings can be extrapolated
to identify the host/ patient characteristics of present-day risk groups for progression to late-stage
syphilis (i.e., presence of PD, LEH, dental cavities, and reduced stature), refining public health
disease screening protocols/initiatives to better prevent late-stage syphilis.

     This project has allowed me to gain valuable research experience. I have participated in
this project as an undergraduate research assistant (URA) since the project's start in winter 2022.
As I am interested in both clinical medicine and public health, participating in long-term research
about syphilis prevention, diagnosis, treatment, and control will allow me to explore both
domains simultaneously. In addition to gaining hands-on training in diverse areas forms of data

collection, database creation and management, and data analysis, I have improved my critical

thinking skills through developing and refining my research design and interpreting my results.

Further, mentorship has been invaluable from Dr. Zuckerman, and her Graduate Research

Assistants (GRA) on the larger project, who are MA students in the Applied Anthropology

program at MSU. Additionally, my professionalization will be greatly enhanced by my inclusion

as a co-author on all outputs from the larger project (e.g., journal articles). I will continue to

participate with the PI and GRAs in data analysis and interpretation, presentation and publication

of results from the larger project and my independent research project, and diverse public and K-

12 outreach and education activities related to the larger project.

References Cited:

Cheverko C, and Hubbe M. 2017. Comparisons of statistical techniques to assess age-related
skeletal markers in bioarchaeology. American Journal of Physical Anthropology
163:407-416.

Marklein KE, Leahy RE, and Crews DE. 2016. In sickness and in death: assessing frailty in
human skeletal remains. American Journal of Physical Anthropology 161(2):208-225.

Partieli, M., & Yiasemis, A. (2021). Ocular Syphilis with HIV Co-infection. *CRO (Clinical &
Refractive Optometry) Journal*, *32*(3), 36821.

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Correlations Between Contamination at CERCLA sites, Income Distribution, and Racial Distribution in Mississippi

Introduction:

The Comprehensive Environmental Response, Compensation, and Liability Act, also known as CERCLA or Superfund, was created because of concern over hazardous wastes being leaked into the environment, and its purpose is to contain, clean, and manage sites of hazardous waste discharge or accidental leakage. The purpose of this research was to determine a spatial link between income, race, and contamination from these sites (per capita). The hypothesis was that as income and/or the percentage of Caucasian citizens in a county increase, the number of these sites would decrease. Previous studies found similar correlations between race, income, and pollution—a study of Charleston, South Carolina linked placement of Underground Storage Tanks to racial distribution (Wilson 2012), and the Detroit Metropolitan Area was found to have lower Toxic Release Inventory emissions in higher-income areas. It is important to note, however, that results when studying the entire state of Michigan were the opposite (Downey 1998). If the results of this study for Mississippi were significant and proved the hypothesis true, these results would have been used to help disadvantaged communities prevent future contamination and fight for safer storage and use of hazardous waste.

The original title of the study was 'Correlations Between Groundwater/Underground Storage Tank (UST) contamination at CERCLA sites, Income Distribution, and Racial Distribution in Mississippi', however it was changed to 'Correlations Between Contamination at CERCLA sites, Income Distribution, and Racial Distribution in Mississippi' just before the Undergraduate Research Showcase after it was decided the title did not accurately enough represent the study.

Methods:

This research was done on a county level in ArcGIS Pro and used available data from the U.S. Census Bureau, the U.S. Bureau of Labor Statistics, and The Mississippi Department of Environmental Quality (MDEQ) GARD database.

The GARD database contains information on Mississippi Underground Storage tanks and CERCLA (also known as Superfund) sites. The number of CERCLA sites with at least one known major contaminant in each county were counted, and these values were divided by each county's total population with the ArcGIS Pro 'calculate field' function to get the final 'CERCLA sites per capita' value.

Population of each county, which was only used for the 'CERCLA sites per capita' value, came from the U.S. Census Bureau, as did the percent of Caucasian citizens. The U.S. Census Bureau also provided TIGER files, which contain basic data for counties in the United States including but not limited

to size, shape, location, and name. Additional fields were added to the data table provided to manually input percent of Caucasian citizens in each county, Average Weekly Income of each county, number of CERCLA sites in each county, population, and finally CERCLA sites per capita (which was calculated, whereas the other represented data came directly from their respective sources). The Average Weekly Income data came from the U.S. Bureau of Labor Statistics and is in U.S. Dollars.

Data: Average Weekly Income (USD)

Weekly Average Income

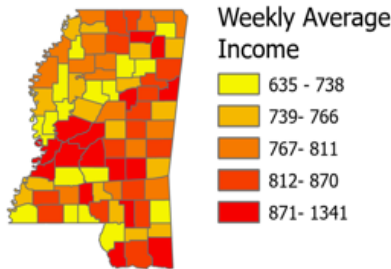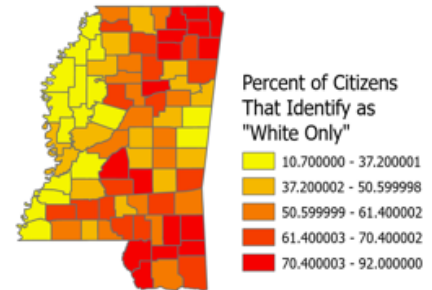- 635 - 738
- 739 - 766
- 767 - 811
- 812 - 870
- 871 - 1341

Fig. 1 (above). Color-coded Map of Mississippi showing Average weekly income in each county.

Data: Percent of Caucasian Citizens

Fig. 2 (right). Color-coded Map of Mississippi showing percentage of Citizens identifying as "White Only" in each county.

Percent of Citizens That Identify as "White Only"

- 10.700000 - 37.200001
- 37.200002 - 50.599998
- 50.599999 - 61.400002
- 61.400003 - 70.400002
- 70.400003 - 92.000000

Data: Number of CERCLA Sites Per Capita

CERCLA Sites Per Capita

- 0.000000 - 0.000141
- 0.000142 - 0.000324
- 0.000325 - 0.000487
- 0.000488 - 0.000745
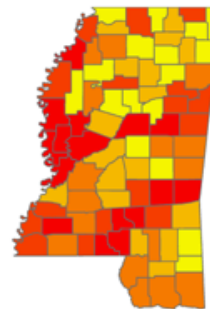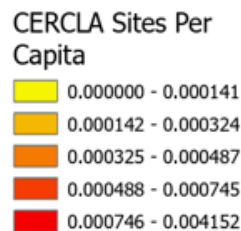- 0.000746 - 0.004152

Fig. 3 (left). Color-coded Map of Mississippi showing Number of CERCLA sites with at least one major contaminant Per Capita in each county.

The data was compiled within ArcGIS Pro, which was then used to create high-quality color-coded maps of the following calculated and raw data: Average Weekly Income in each county, percentage of Caucasian citizens in the county (represented by 'Percent of Citizens that Identify as "White Only"'), and CERCLA sites per capita in the county. Hotspot Analysis, a tool within ArcGIS Pro, was used to locate areas of the state where higher or lower values in these datasets are clustered. It finds areas where a county with a higher value is surrounded by other counties with higher values, and a county with a lower value by other counties with lower values. Figure 5, for example, illustrates that lower percentages of Caucasian citizens (ie. lower values) are clustered in the western part of Mississippi.

Data: Average Weekly Income (USD)

Hotspot Analysis

- Cold Spot with 99% Confidence
- Cold Spot with 95% Confidence
- Cold Spot with 90% Confidence
- Not Significant
- Hot Spot with 90% Confidence
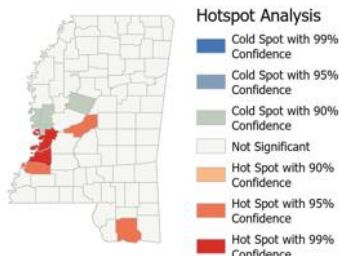- Hot Spot with 95% Confidence
- Hot Spot with 99% Confidence

Fig. 4 (above). Color-coded Map of Mississippi showing Clusters of high and low Average Weekly Income in Mississippi Counties. (Hotspot Analysis)

Data: Percent of Caucasian Citizens

Hotspot Analysis

- Cold Spot with 99% Confidence
- Cold Spot with 95% Confidence
- Cold Spot with 90% Confidence
- Not Significant
- Hot Spot with 90% Confidence
- Hot Spot with 95% Confidence
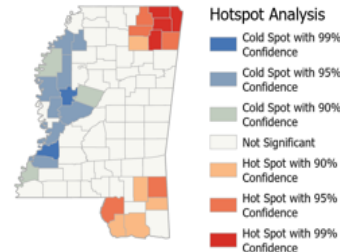- Hot Spot with 99% Confidence

Fig. 5 (above). Color-coded Map of Mississippi showing Clusters of high and low Percentages of Caucasian Citizens in Mississippi Counties. (Hotspot Analysis)

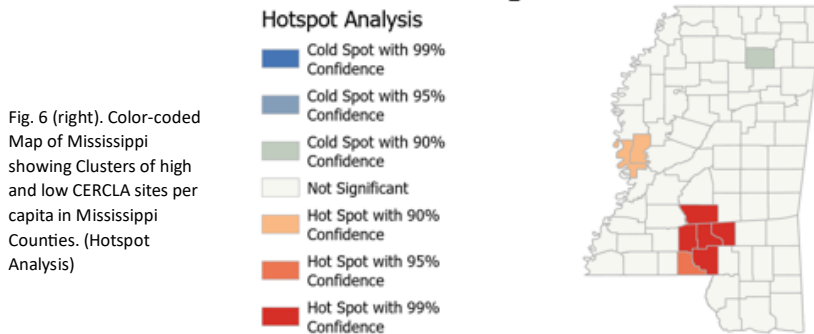## Data: Number of CERCLA Sites Per Capita

Fig. 6 (right). Color-coded Map of Mississippi showing Clusters of high and low CERCLA sites per capita in Mississippi Counties. (Hotspot Analysis)

Final Results and Discussion:

An R-squared value is used to represent how random or how significant the correlation between separate datasets is. A value closer to one indicates a very close relationship, and a value closer to zero indicates little to no relationship. The R-squared values for this study were also calculated within ArcGIS Pro. The relationship between Race and number of CERCLA Sites Per Capita is present as it was predicted, however the significance of this relationship is very small with an R-squared value of only 0.07. The correlation between Income and CERCLA Sites Per Capita is also present in the way hypothesized—as income increases, Sites Per Capita decrease—however the significance is even less, with an R-squared value of just 0.01.

Fig. 7 (right). Histogram showing the correlation between percentage of Caucasian citizens and CERCLA sites Per Capita in Mississippi Counties.
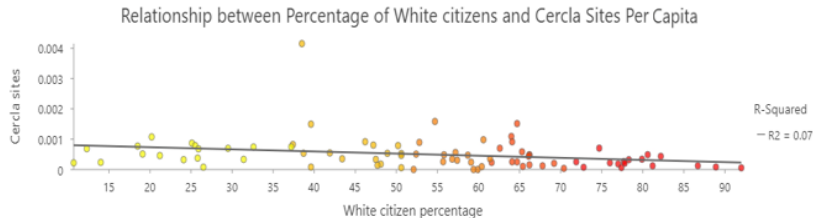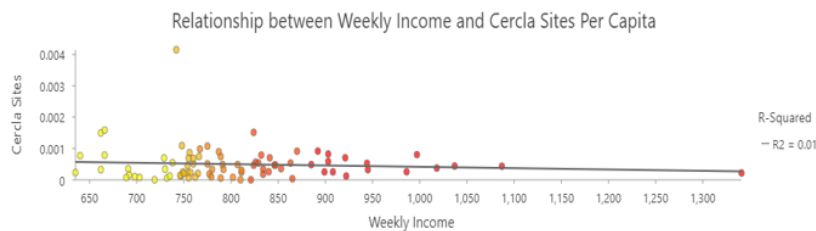

Fig. 8 (right). Histogram showing the correlation between Average Weekly Income and CERCLA sites Per Capita in Mississippi Counties.

Conclusions:

Considering the low R-squared values for the correlation between Race and Income and CERCLA sites per capita, the results of this study can be stated as follows:

- On a state-wide level, the correlation between Race and the number of CERCLA sites per capita in Mississippi Counties is not significant.
- On a state-wide level, the correlation between Income and the number of CERCLA sites per capita in Mississippi Counties is not significant.

One reason for these results could be the distribution of CERCLA sites, Income, and Race within individual counties—for example, the northwestern corner of a county could have a very high income while the rest of the county has a very low income, however the data used in this study was averaged to fit entire counties. Even within individual cities, there are clusters of like incomes, neighborhoods where primarily minority citizens live, and neighborhoods where primarily Caucasian or "white" citizens live. This was the most likely reason the results of the previously referenced studies were significant while this study's results were not. If this study were to be repeated or built upon, it would most likely be done on a much smaller scale—within one single city or one single county. A smaller scope of study allows for a more in-depth analysis of CERCLA site placements and a better understanding of how Race and Income can be used to predict placement of CERCLA sites in Mississippi.

References:

1. Wilson, S., Fraser-Rahim, H., Zhang, H., Williams, E., Samantapudi, A., Ortiz, K., Abara, W., & Sakati, W. (2012). The spatial distribution of leaking underground storage tanks in Charleston, South Carolina: An environmental justice analysis. *Environmental Justice, 5* (4), 198-205. https://doi.org/10.1089/env.2012.0011

2. Downey, L. (1998). Environmental Injustice: Is Race or Income a Better Predictor? *Social Science Quarterly*, *79*(4), 766–778.   http://www.jstor.org/stable/42863846