Ant Biodiversity Within the Holtsville Wildlife and Ecology Center Serving as an Indicator of Environmental Pollutants

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ABSTRACT

The purpose of this study is to examine the physical and genetic variations of ants at a former landfill, the Holtsville Wildlife and Ecology Center (HWEC), to infer if the biodiversity of these organisms, and thus the site, has been influenced by potential pollutants present at the center that could harm humans. Methods of phenotypic and genotypic analysis of the ants will be used to understand species diversity in the former landfill. Ants are known as an indicator species, making them viable organisms for use in this study and to view the impact of pollutants on human health. It is hypothesized that the presence of pollutants at the HWEC will be signaled by the low biodiversity of ants at the different collection locations since biodiversity corresponds to ecosystem stability. Ants were collected from three distinct locations at the HWEC. Out of the twenty-seven ants collected, twenty were selected to be analyzed. Using a silica extraction method, DNA was isolated and sequenced from these specimens. After this sequencing, ten samples were removed from consideration due to poor results. Sequencing the ants from site 1 resulted in the species *Tetramorium caespitum* to be found. Ants from site 2 were the most diverse, containing the species *Lasius alienus* and *Lasius neoniger*. At site 3, ants returned as the species *Tetramorium immigrans*. Considering the entire former landfill's biodiversity, the presence of four distinct species of ants suggests a high HWEC biodiversity, indicating overall stability. Since approximations of this stability came from ant biodiversity measurements and ants are described as excellent bioindicators, it is defended further, supporting the conclusion that there are no harmful pollutants in the center. This refutes this paper's hypothesis, relieving concerns that the status of this specific center (HWEC) as a landfill-turned-park could cause human health issues, and illustrating that ant biodiversity can be indicative of pollutant presence at former landfills.

INTRODUCTION

The status of ants as an indicator species, a species that is representative of the potential effects of the conditions of the environment it lives in, means these organisms are favorable selections for viewing the impact of pollutants and harmful substances on human health. The HWEC in Holtsville, New York, is a large park in the Town of Brookhaven. Once a landfill, the area was rehabilitated by the local highway department after the landfill's closure in 1974 (Town of Brookhaven, NY, n.d.). Former landfills, like the HWEC, have been documented by various researchers as sites where environmental pollutants may still be present, with such pollutants including heavy metals, leachate, nitrate, chlorides, and coliforms (Burlakovs & Vircavs, 2012; de Rosa et al., 1996; Díaz Rizo et al., 2011). Further, analyzing ant biodiversity is supported by researchers to achieve strong measurements of an ecosystem's stability (Loreau & De Mazancourt, 2013; Tiede et al., 2017). This analysis can be completed phenotypically and genotypically, with phenotypic analysis being done by examining the physical characteristics of ants for markers of pollution, such as specific variations in coloring or size, and genotypic analysis being completed via DNA Barcoding, a method of DNA analysis that uses information within a single gene region common across all taxa and accesses that information by DNA sequencing under universal conditions (Armstrong et al., 2005; Grześ et al., 2014; Skaldina et al., 2018). With these ideas in mind, this research attempts to study the impact potential pollutive substances have on ant biodiversity measurements at the HWEC, thus indicating the environment's biodiversity, and answering the following research question: To what extent does the biodiversity of ants, an indicator species, reflect the presence of pollution and harmful substances in a former landfill?

MATERIALS & METHODS

Specimen Collection

Twenty-seven samples were collected from three locations at the HWEC using Keebler Pecan Sandies Cookies as bait. Ants found were placed in small containers and euthanized in a freezer. Site 1 was a composting center, site 2 an exercise pathway, and site 3 a pathway for construction vehicles.

Extraction & Amplification of DNA

Seven samples with poor photo documentation were not selected for extraction of DNA, which was completed using a silica extraction technique. The DNA of the remaining twenty samples, with roughly seven being from each site, was amplified through PCR with the COI primer, and the amplified DNA was then analyzed through gel electrophoresis and sent for sequencing.

Specimen Analysis

The MUSCLE sequence alignment software present in the DNA Subway Program enabled the matching of ten samples that were sequenced without error to a corresponding species in the BLAST database. After the matching of sequences to species, the genetic data was examined to draw conclusions on the biodiversity of the ants, the biodiversity of each collection site, and the HWEC's stability.

RESULTS

Figure 1. Map of HWEC: The HWEC consists of several features that make it a unique location to perform research, but its status as a former landfill stands out as its most attractive aspect. Specifically, Site 1, the composting center, is another attractive aspect, as studies have shown that pollution present at composting centers could be intensified by composting practices (Chu et al., 2019).

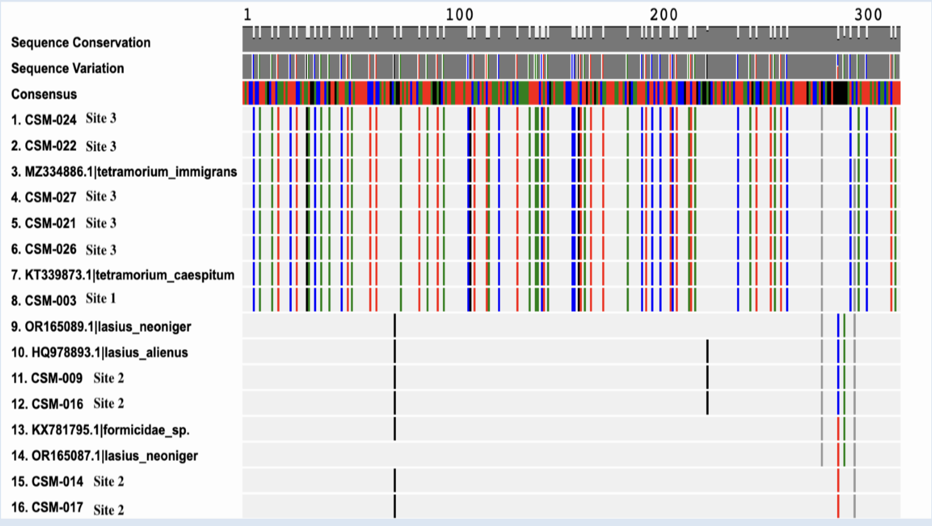

Figure 2. Data received from MUSCLE: The MUSCLE sequence alignment software was used to match similar DNA sequences to each other. At site 1, the composting center, specimens matched to the species *Tetramorium caespitum*; at site 2, the exercise pathway, to *Lasius alienus* and *Lasius neoniger*; and at site 3, the construction pathway, to *Tetramorium immigrans*.

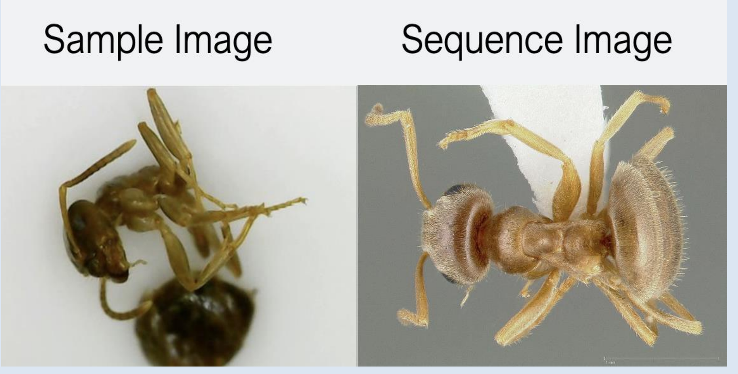

Figure 3. Sample CSM-014 from Site 2 (left) and the database image for sequence OR165087.1|lasius_neoniger (right): Interestingly, after sequence alignment, the two samples from site 2, the exercise pathway, that matched with the species *Lasius neoniger* had two base-pair variations with this species.

DISCUSSION

The results of this study indicate that site 2, the exercise pathway, was the most biodiverse within a single site, containing ant specimens from two distinct species of the same genus (*Lasius alienus* and *Lasius neoniger*), thus showing the greatest stability (Loreau & De Mazancourt, 2013; Tiede et al., 2017). Human foot traffic, present at most at site 2, could have influenced this finding, so more research should be conducted to test this discovery specifically. Furthermore, the two base-pair variations in the sequencing for specimens CSM-014 and CSM-017, which were linked to the species *Lasius neoniger*, raise some concerns as to the accuracy of their correlation. These variations occur due to the incompleteness and lack of accuracy of molecular reference databases, hindering the match between these specimens from site 2 and the *neoniger* species (Pawlowski et al., 2018). This limitation lessens support for site 2 being a location of stability, with the variations challenging the site's true biodiversity, then causing the strength of a conclusion regarding its stability to decrease. At site 1, the composting center, only one specimen, CSM-003, was returned after sequencing all samples. This means that biodiversity within the site cannot be measured as there are no other correctly sequenced site 1 samples to compare to CSM-003. Consequently, further studies should be conducted at sites like the composting center, as it presents itself as an intriguing location for ant biodiversity research due to its connection to possible pollution. Moreover, at site 2, specimens CSM-009 and CSM-016 had lighter coloring at their thoraxes and abdomens compared to their matching sequence image, with a similar head color. This difference is important to point out, but no studies in the collected body of knowledge tell of what the lighter coloring of these specific ant regions could indicate, while other studies have stated that lighter coloring of the head is indicative of heavy metal pollution (Skaldina et al., 2018). This may still indicate pollution of this site, but further research should be conducted to measure the possibilities of the coloring's indications. Additionally, physical characteristics of the ants could not be compared effectively due to differences in quality and scale between photo documentation of specimens. Thus, more research should be conducted with increased accuracy in photo documentation, allowing for these comparisons. Considering the entire former landfill's biodiversity, the presence of four distinct species of ants suggests a high HWEC biodiversity, indicating overall stability. Since approximations of this stability came from ant biodiversity measurements and ants are described as excellent bioindicators, it is defended further, supporting the conclusion that there are no harmful pollutants in the center. This refutes this paper's hypothesis, relieving concerns that the status of this specific center (HWEC) as a landfill-turned-park could cause human health issues, and illustrating that ant biodiversity can be indicative of pollutant presence at former landfills.

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