Earhart DNA Research Update

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At the request of The International Group for Historic Aircraft Recovery (TIGHAR), University of Oklahoma researchers have been evaluating a bone fragment excavated from an archaeological site on Nikumaroro (formerly Gardner Island). TIGHAR is testing the hypothesis that Amelia Earhart died as a castaway on the uninhabited Pacific atoll. The bone fragment's structure and the context in which it was found have led TIGHAR to wonder if it might be part of a human finger.

TIGHAR also asked OU researchers to evaluate small clumps of material recovered from the same archaeological site to determine whether they might be human fecal matter.

At this time the analyses of the TIGHAR samples are inconclusive. We are providing this update in recognition of the high level of public interest in the outcome of this investigation.

The Bone Fragment

We were not able to retrieve sufficient DNA from the bone sample to be able to provide any definitive statements on the bone's origin.

The bone fragment was very small, approximately one gram of material. Following appropriate ancient DNA protocols, we attempted to extract DNA from .25 grams of the material. We used a real time Polymerase Chain Reaction method (real time PCR or rtPCR) to detect human mitochondrial DNA in the extract. Two of these rtPCRs provided a positive result. However, during quality control protocols, we were unable to repeat this result with additional rtPCRs. This suggests that either 1) the initial detection of human DNA was attributed to a sporadic DNA contamination event, and in reality, there was no usable human DNA preserved in the extract, 2) there was human DNA in the extract, but it was too little, or of too poor of quality, to consistently analyze, 3) DNA in the bone was non-human. A second DNA extraction also failed to provide positive results for human DNA.

Because the bone is clearly from an animal, human or otherwise, additional PCRs were used to detect animal DNA more generally. These PCRs provided no positive results. The fact that these PCRs were unsuccessful suggest that either 1) there is no animal DNA in the bone, 2) there was animal DNA in the extract, but it was too little, or of too poor of quality, to reliably analyze, 3) the PCR design was ineffective for targeting the particular animal.

Approximately 0.5 grams of bone material remained after our study. For posterity, we have decided to preserve this remaining bone. Genome technologies are developing at a rapid pace. To what extent ancient DNA research will benefit from these developments remains to be seen. Nevertheless, there is reason for optimism that some day in the near future, less destructive, and more sensitive genomic methods will be able to resolve the bone's origin. For now, the question of whether the bone is human must remain unanswered.

The Soil or Fecal Clumps

Genetic testing of the soil or fecal clumps remains an ongoing investigation.

TIGHAR provided OU researchers with approximately 4 grams of unknown clumps of material resembling soil or feces. Archaeologically, the clumps were associated with human activity and are anomalous in the context of the site. The clumps' physical characteristics (morphology) were examined by University of Maine researcher

who has extensive experience in analyzing ancient fecal material. The researcher concluded the mass had some fecal properties. Our objective was to further evaluate the clumps using molecular genetic methods.

Modern DNA contamination was a concern because the clumps were directly handled by excavators. Within an ancient DNA clean room environment, the outer surface of one sample was removed. The remaining mass was submerged in concentrated commercial bleach to destroy any residual modern DNA contamination. This procedure has been effective in other ancient DNA studies of feces. Following the bleach procedure, two successful DNA extractions were performed, each on .25 grams of material. Initially, we used a PCR based method, which successfully detected human DNA and DNA typical of bacteria found in an animal's gut, *Atopobium sp* and *Enterococcus sp*. This suggests that the sample was fecal material. However, these bacteria can be found in some soils.

Currently, we are using a genetic method to more broadly examine bacteria species within the material. Very preliminary results are detecting soil bacteria typical of an island, but these results are far from conclusive and the material was recovered from an area on the island devoid of soil. In the near future, we will be using a next-generation DNA sequencing technology to provide a more in-depth examination of the microbes.

Sequencing of the human DNA resulted in two different sequences but, so far, not enough human DNA has been extracted and sequenced to match against reference samples. The most common explanation for multiple sequences is either the sample is associated with a temporary latrine used by more than one person, or the retrieved data still includes modern human contamination. We will continue to explore how well these explanations fit the data by further molecular testing.

In addition to the bacteria and human DNA analyses, future analysis will include targets for plants and animals. The presence of certain plant and animal DNA would be a further indication that the clumps are fecal matter and could provide information about the diet and general health of the individual. Preliminary chemistry for sequencing plant DNA was successful. At this time, we do not foresee any problems targeting animal DNA, with a particular interest in non-human animals. This process is likely to take three months.