INTRODUCTION

Human behavior has effected non-human morphological evolution in many ways, including non-human body size evolution due to size-selective human hunting/harvesting pressures. Intertidal mollusc exploitation by humans has been well-documented throughout the archaeological record due to the trash heaps (middens) of shells left after processing. These middens might also provide: evidence of prey phenotypic change in response to these harvesting pressures; potential opportunities to recover temporal genetic records, confirm a genetic basis for the phenotypic change, and powerfully test adaptive hypotheses.

Size at sexual maturity in Bocas del Toro West Indian fighting conch (Strombus pugilis) has decreased consecutively from the paleontological “pre-human” period to the present day. This size decrease was associated with a decline in edible meat weight by ~40% over the past 7,000 years. We revisited these sites/collections (see Figure 1 and map of Bocas del Toro with the following map:

1. Build a reference genome sequence for S. pugilis from a fresh tissue sample
2. Develop a method to extract both modern and ancient DNA from discarded marine snail shells

SAMPLE PREPARATION

Live-caught snails were relaxed and pulled from their shell. A thin disk of tissue was taken from each snail’s foot and preserved (see above). ~30 mg of tissue per individual was used for OMEGA E.Z.N.A. Tissue Kit extraction. Shells were frozen.

Mechanical processing was used to maximize the recovery of DNA within the calcium carbonate shell matrix (as shown above). ~1 g of shell powder per individual was digested for a total of 48 hours, then DNA was purified with a modified version of the Qiangen QIAquick PCR Purification Kit. Archeological and paleontological shells were processed in an ancient DNA facility.

REFERENCE GENOME ASSEMBLY & SHELL DNA RECOVERY

The tissue sample from Cayo Agua 2.3 was sequenced for the reference genome, with 47.33 X average sequencing depth across the assembly. The total length of the Cayo Agua 2.3 reference assembly is 1.54 Gb. We are working to annotate and improve the quality of the reference assembly. Our next steps include assessing patterns of expected ancient DNA damage in reads mapping to the mitochondrial genome, where sequence coverage is expected to be higher than the nuclear genome. Ultimately, based on these successful preliminary results, we expect that through deeper sequencing of the shell libraries and/or the addition of DNA capture methods prior to sequencing, it will be possible to reliably genotype nuclear genome single nucleotide polymorphisms (SNPs) from the better preserved ancient DNA samples.

CONCLUSIONS & FUTURE DIRECTIONS

O’Dea et al. characterized a morphological change (i.e. size at sexual maturity) due to a human behavior (long-term, low-level harvesting). With the ability to extract and sequence DNA from both modern and ancient shells, we could potentially:

- Identify genomic regions that are associated with body size variation (GWAS)
- Incorporate ancient DNA for direct evidence of allele frequency change over time

We are continuing to develop the methods discussed here, and our next priority for this project will be sequencing the rest of the tissue and shell samples (see Figure 1). Future sampling could be expanded to a larger population scale for GWAS and population evolutionary genomic analyses, and could include other sites in the Bocas del Toro archipelago, including areas of lower-intensity harvesting where the S. pugilis seem to have slightly larger body sizes. It is our goal that developing these methods will allow us to quantify evolutionary morphological changes due to human behavior and how quickly such changes can occur.

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