Elephant Species Identification from Ivory through Polymerase Chain Reaction and Sequencing Analysis for Application in Works of Art

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Introduction

- Ivory is a tooth that protrudes out of an animal's mouth. It is the hard, white material that makes up the tusks of elephants and other animals. It has been used for centuries in medicines, weapons, and art. In China today, ivory is seen as a status symbol and is in high demand despite how lethal ivory harvesting is to animals.
- Ivory has primarily come from elephants. There are three main species: African Bush Elephant (*Loxodonta africana*), African Forest Elephant (*Loxodonta cyclotis*), and Asian Elephant (*Elephas maximas*)

Flora, African Elephant (Vulnerable) Shirley, Asian Elephant (Endangered) Elephant Sanctuary in Tennessee

- There is no known way to differentiate ivory from African and Asian elephant species morphologically
- Ivory species identification is currently important for many reasons:
  - Art exhibitions are required to identify the ivory source's species in order to get through customs. CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) laws have been more strictly enforced, necessitating species identification.

Left: *Diptych Leaf With Scenes of the Passion* (Master of the Kremsmünster Diptych, The Walters Art Museum, Baltimore)

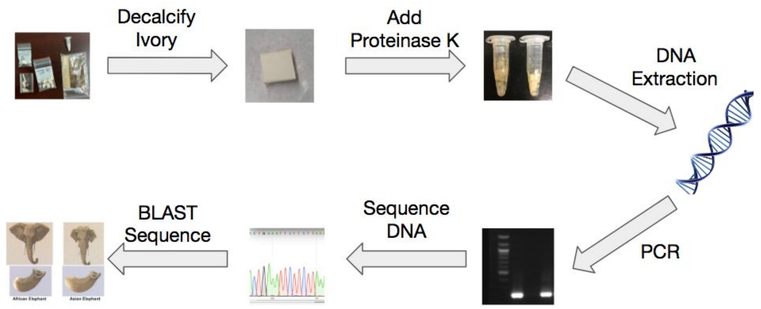
Right: *Nativity (*Byzantine, The Walters Art Museum, Baltimore)

  - Knowledge of the species will provide information that places the ivory object in physical, historical, and cultural context.
  - Elephants are being poached for their ivory and species testing assists in the investigations of illegal trade, helping endangered species survive.
- Mitochondrial DNA (mtDNA) is DNA contained in the mitochondria separate from genomic DNA. Using known changes in the DNA, single nucleotide polymorphisms (SNPs) and previous procedures, it is possible to determine the species of elephant a sample of ivory belongs to.[1]

Hypothesis

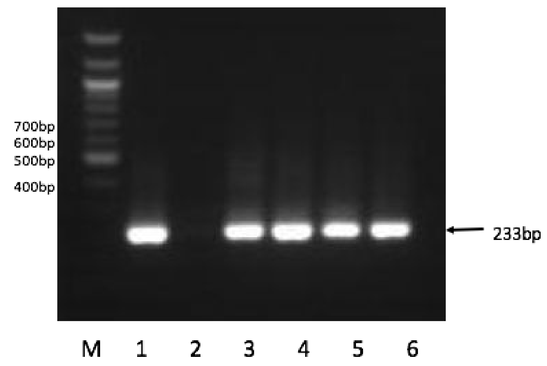
- Using known SNPs in mtDNA, we will be able to determine whether an ivory sample is from an African or Asian elephant species by extracting and analyzing mtDNA.
- We wish to test if current genetic methods of ivory identification are suitable to art conservation science studies.

Methods



PCR Results

Successful PCR Amplification of Ivory mtDNA (below)
Bands containing amplified mtDNA fragments confirm success of PCR with bands appearing at ~200bp. M=Ladder, 1-6 Ivory Samples with Primer Set 4. Lane 2 Blank due to researcher error.



References:
1. Kitpipit, Thitika, et. al. "Mini-SNaPshot Multiplex Assays Authenticate Elephant Ivory and Simultaneously Identify the Species Origin." *Forensic Science International: Genetics* 27 (2017): 106-15.
2. *Flora*. Digital image. *The Elephant Sanctuary in Tennessee*. Web. https://elephants.com/elephants/flora
3. *Shirley*. Digital image. *The Elephant Sanctuary in Tennessee*. Web. https://elephants.com/elephants/flora

Sequencing Results

Loxodonta cyclotis isolate DS1511 mitochondrion, complete genome
Sequence ID: KY616976.1 Length: 16010 Number of Matches: 1

Range 1: 15357 to 15523 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
300 bits(162)	1e-77	165/167(99%)	0/167(0%)	Plus/Plus

```
Query  17     GGNAGAGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTC  76
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  15357  GGAAGAGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTC  15416

Query  77     CCTGCAAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTTGTCCCCATACATAA  136
              ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  15417  CCTGCAAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTCGTCCCCATACATAA  15476

Query  137    TGATATATATTACTAACTATACTTAATCTTACATAGACCATACTATG  183
              ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  15477  TGATATATATTACTAACTATACTTAATCTTACATAGACCATACTATG  15523
```

Loxodonta cyclotis isolate DS1511 mitochondrion, complete genome
Sequence ID: KY616976.1 Length: 16010 Number of Matches: 1

Range 1: 15357 to 15532 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
313 bits(169)	1e-81	173/176(98%)	0/176(0%)	Plus/Plus

```
Query  18     GGNAGAGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTC  77
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  15357  GGAAGAGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTC  15416

Query  78     CCTGCAAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTTGTCCCCATACATAA  137
              ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  15417  CCTGCAAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTCGTCCCCATACATAA  15476

Query  138    TGATATATATTACTAACTATACTTAATCTTACATAGACCATACTATGTNATAATCGT  193
              ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  15477  TGATATATATTACTAACTATACTTAATCTTACATAGACCATACTATGTATAATCGT  15532
```

Loxodonta cyclotis isolate DS1511 mitochondrion, complete genome
Sequence ID: KY616976.1 Length: 16010 Number of Matches: 1

Range 1: 15362 to 15535 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
302 bits(163)	3e-78	170/174(98%)	1/174(0%)	Plus/Plus

```
Query  24     AGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTCCCTGC  83
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  15362  AGGTCCACTTACCTCGCTATCAATACCCAAAACTGAAATTCTTCTTAAACTATTCCCTGC  15421

Query  84     AAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTTGTCCCCATACATAATGATA  143
              ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  15422  AAGCAAACCAACCCGCTATGTATATCGTGCATTAAATGCTCGTCCCCATACATAATGATA  15481

Query  144    TATATTACTAACTATACTTAATCTTACATAGACCATACTATGN-TNATCGTGCA  196
              |||||||||||||||||||||||||||||||||||||||||| | ||||||||
Sbjct  15482  TATATTACTAACTATACTTAATCTTACATAGACCATACTATGTATAATCGTGCA  15535
```

Ivory mtDNA Can Determine Ivory Origin
BLAST alignments of ivory-extracted mtDNA determining the ivory to be of African origin. Alignment was not specific enough to give species, only genus.

Conclusions

Based on our methods and results, we believe that current genetic species identification techniques are ill-suited to the identification of ivory samples for art works. The sample size required for these techniques would prove disfiguring and destructive to the artwork, making this technique infeasible. If smaller sample sizes could be taken, and mtDNA extracted, then this method could theoretically be viable.

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